

# Supplementary Information

## **Functional expression of TLR5 from different vertebrate species and diversification towards intestinal pathogen recognition**

Eugenia Faber <sup>1</sup>, Karsten Tedin <sup>2</sup>, Yvonne Speidel <sup>1</sup>, Melanie M. Brinkmann <sup>3</sup>,  
Christine Josenhans <sup>1,4\*</sup>

<sup>1</sup> Medizinische Hochschule Hannover, Institute for Medical Microbiology, Carl-Neuberg-Strasse 1, 30625 Hannover, Germany

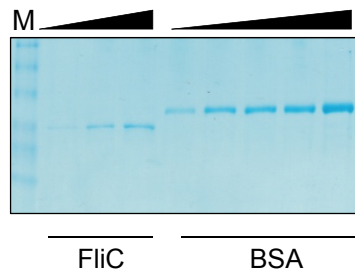
<sup>1</sup> DZIF-German Center for Infection Research, Partner site Hannover-Braunschweig, Germany

<sup>2</sup> Institute of Microbiology and Epizootics, Free University Berlin, Germany

<sup>3</sup> Helmholtz Center for Infection Research, Braunschweig, Germany

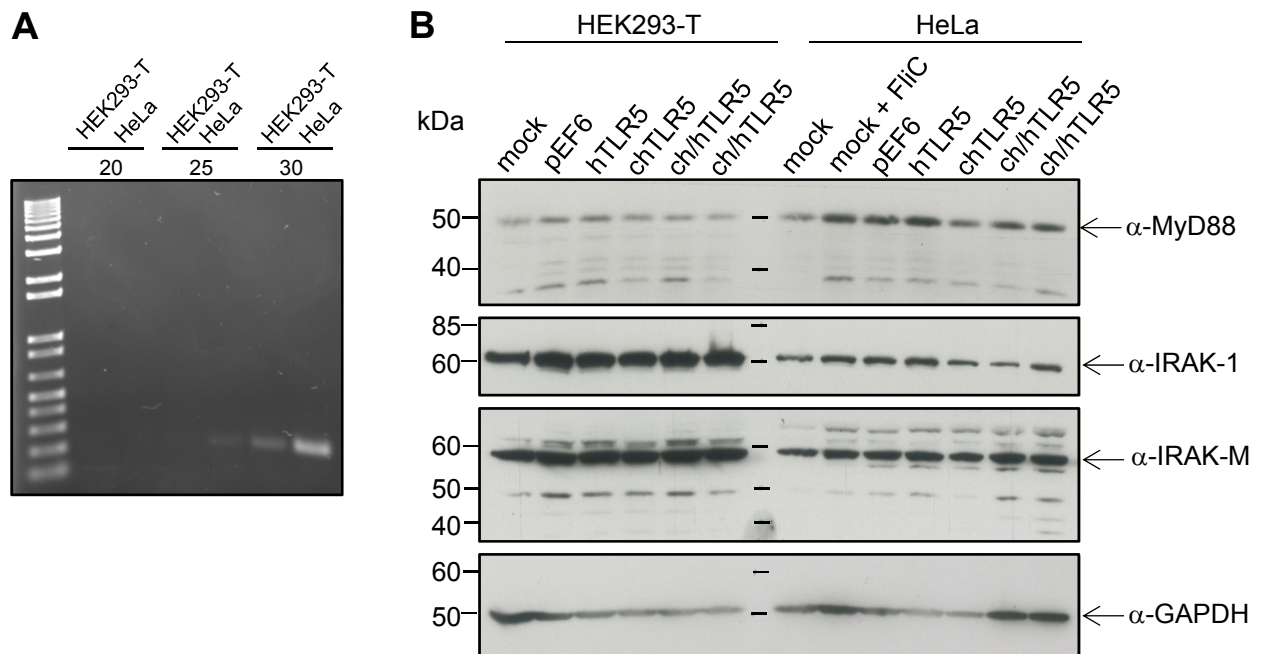
<sup>4</sup> Max von Pettenkofer Institute, Ludwig Maximilians University Munich, Pettenkoferstrasse 9a, 80336 Munich, Germany

\*Address correspondence to:  
Christine Josenhans; [Josenhans@mvp.uni-muenchen.de](mailto:Josenhans@mvp.uni-muenchen.de)



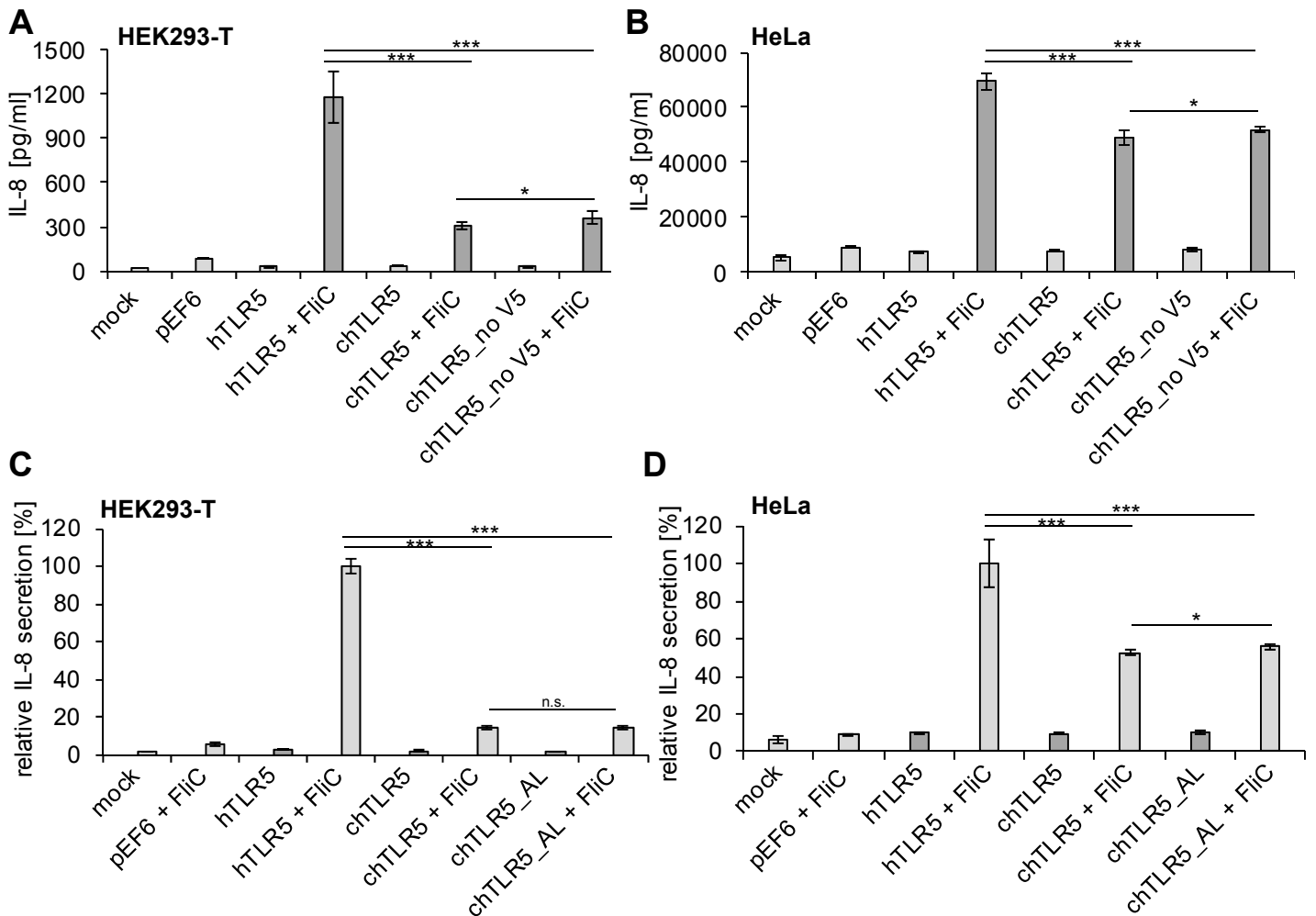
**Faber *et al.*, Figure S1**

**Fig. S1: determination of protein amount and purity of highly purified recombinant *S. Typhimurium* FliC.** Different amounts of recombinantly expressed and highly purified *S. Typhimurium* FliC (used as reference protein for TLR5 activation) were loaded on an SDS gel and stained with Simply Blue Safestain (Novex): M: protein molecular mass standard (Bench Mark Prestained, Novex), lane 1 from left: 20 ng FliC; lane 2: 100 ng FliC, lane 3: 200 ng FliC, lane 4: 100 ng BSA, lane 5: 200 ng BSA, lane 6: 300 ng BSA, lane 7: 500 ng BSA, lane 8: 1 µg BSA.



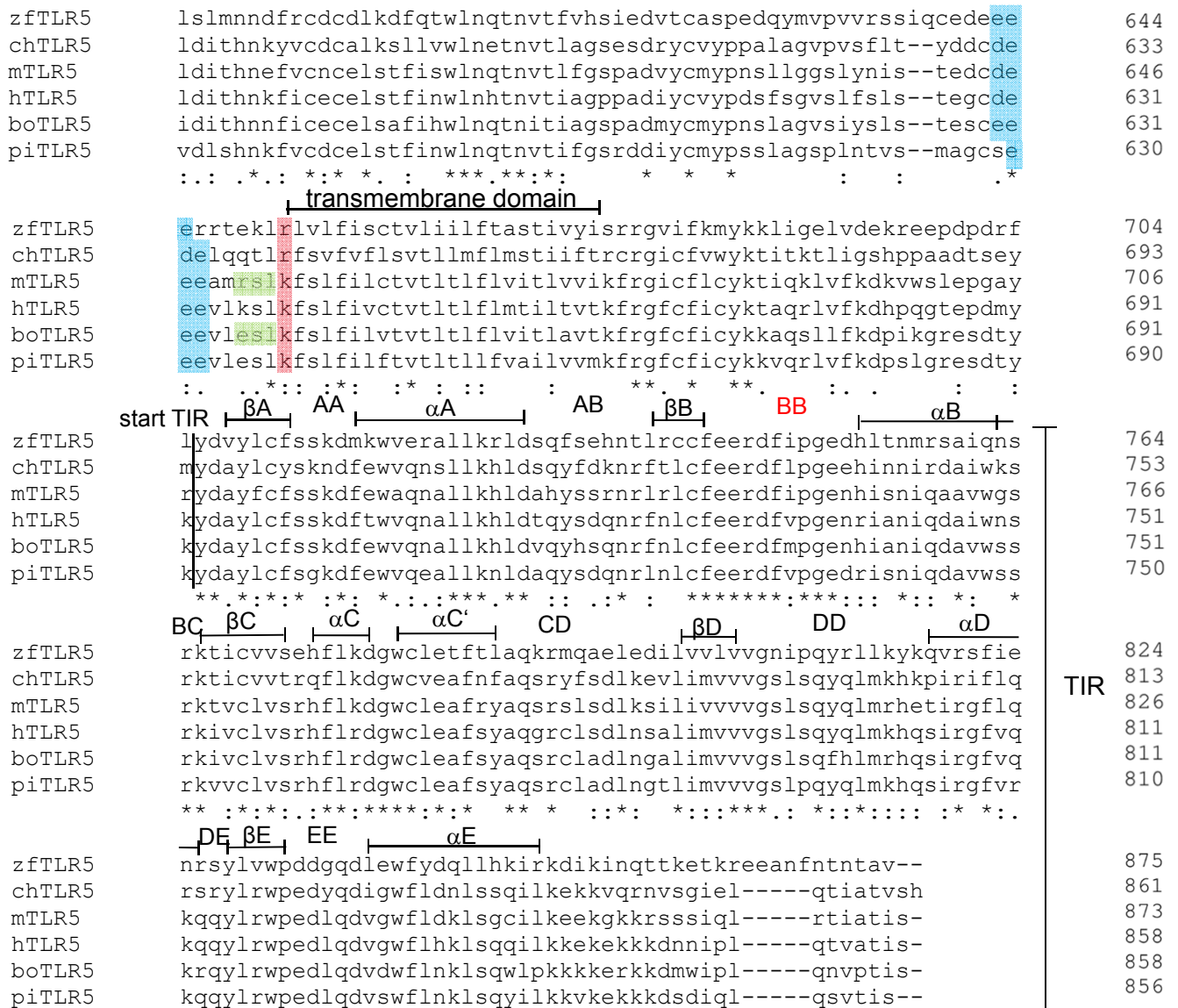
Faber *et al.*, Figure S2

**Fig. S2: comparative expression of TLR signaling pathway components in HEK293-T and HeLa cells.** **A)** Semiquantitative PCRs for MyD88 transcript were performed using cDNA of HEK293-T or HeLa cells as template and the following primers: hMyD88\_F1 and hMyD88\_R1. 5  $\mu$ l of the amplified PCR product (after 20, 25 or 30 amplification cycles) was loaded on a 1% agarose gel. **B)** Western blot analysis using cleared lysates of empty vector (200 ng) or TLR5-V5 construct-transfected HEK293-T (h: 75 ng, ch: 200 ng, ch/h: 200 ng and 250 ng) or HeLa cells (h: 50 ng, ch: 500 ng, ch/h: 50 ng and 100 ng) using anti-MyD88, anti-IRAK-1, anti-IRAK-M and anti-GAPDH antibodies (see Table S6).



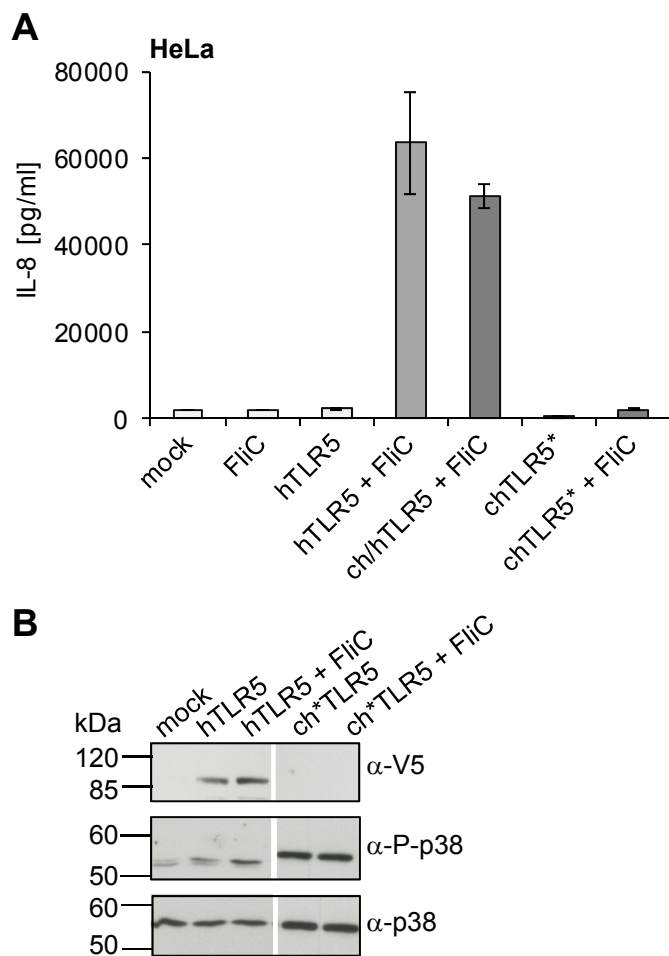
**Faber et al., Figure S3**

**Fig. S3: C-terminal V5-tag or an alternative 3' upstream sequence have minor influence on the activation efficiency of expression constructs for chicken TLR5 by *Salmonella* FliC.** HEK293-T (A, C) or HeLa cells (B, D) were transiently transfected with empty vector pEF6-V5: 200 ng; human TLR5-V5: 100 ng; chicken TLR5-V5 or same construct without V5 tag: 200 ng. For comparison, expression constructs for chicken TLR5-V5 with alternative upstream (AL) sequences were analyzed. 48 h post transfection, cells were coincubated with purified recombinant *Salmonella* FliC (50 ng/well), followed by a measurement of secreted IL-8 in cell supernatants. C and D: IL-8 secretion of hTLR5-transfected and FliC-activated cells was set to 100 % (reference); relative IL-8 secretion of other constructs with regard to the reference is depicted in [%]. Mean and standard deviation from technical triplicates of biological duplicates of a representative experiment are shown. Significant differences between FliC-stimulated hTLR5 and activated chicken TLR5 variants as well as between activated chTLR5 and activated mutated chTLR5 variants are indicated by asterisks (Student's *t*-test, unpaired, two-tailed) as follows: \*, 0.01<*p*<0.05; \*\*, 0.001<*p*<0.01; and \*\*\*, *p*<0.001, n.s. non-significant.



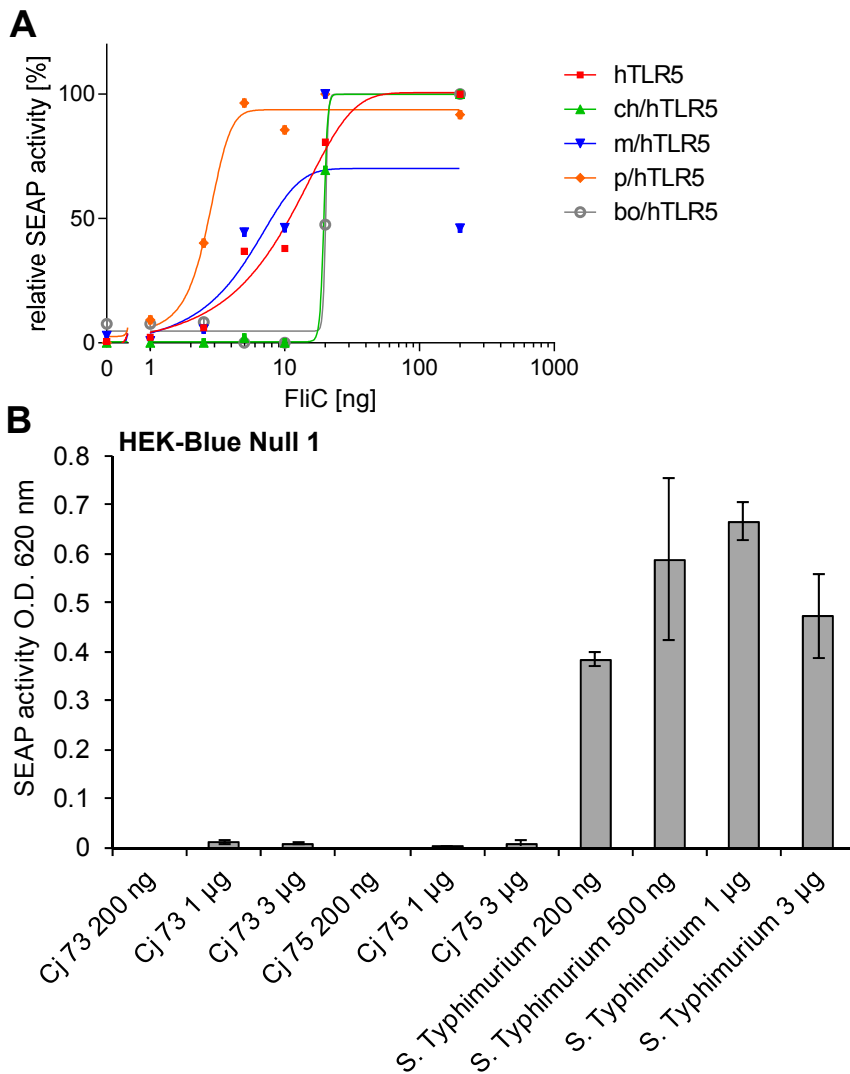
### Faber *et al.*, Figure S4

**Fig. S4: alignment of partial TLR5 amino acid sequences (focus and transmembrane and intracellular domains) from different vertebrate species.** Amino acid sequences of TLR5 receptors from different vertebrate species (zf: zebrafish, ch: chicken, m: murine, h: human, bo: bovine, pi: porcine) were aligned using ClustalW2 (Larkin *et al.*, 2007). Structural components of intracellular TIR domains were assigned according to (Xu *et al.* 2000) and are depicted as abbreviations above the alignment. These components include the BB loop (BB in red) which is involved in TIR-TIR interactions. The start residue of the C-terminal human segment after the non-human N-terminal ECD domain within the chimeric receptor constructs is marked in red. Amino acid exchanges in chimeric mouse/human and bovine/human TLR5 generated in this study by the cloning strategy are marked in green and are as follows: m/h: RSL to TGT and bo/h: ESL to SGT. Acidic residues in the linker domain upstream of the transmembrane domain, which are most likely important for the interaction of TLR5 with UNC93B1 (as shown for mouse UNC93B1 and murine nucleotide-sensing TLRs (Kim *et al.*, 2013) and for proper TLR5 functionality (Huh *et al.*, 2013)), are not affected by the cloning strategy of the chimeric proteins and are shaded in blue.



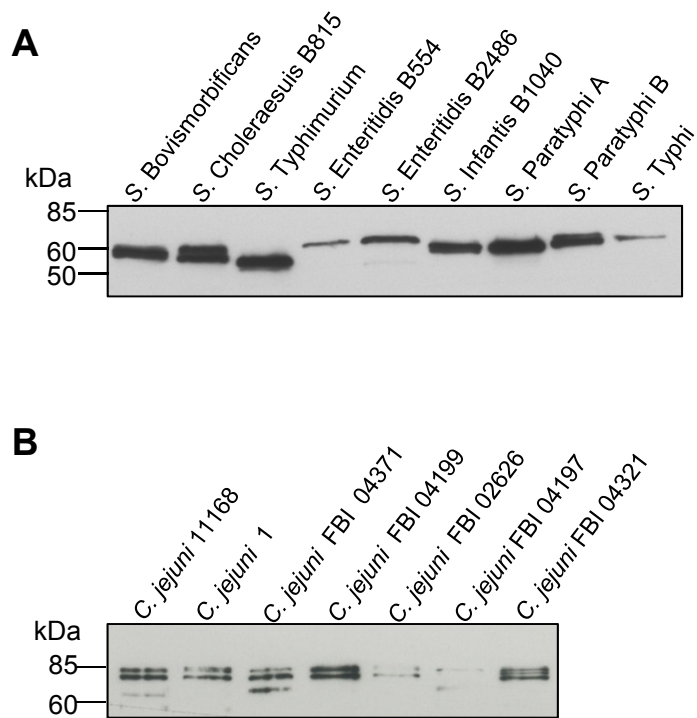
**Faber et al., Figure S5**

**Fig. S5: mutation of amino acid 744 in the chicken TIR domain to human counterpart results in abolished expression of receptor.** HeLa cells were transiently transfected with human (h) TLR5-V5: 100 ng; chicken/human chimeric (ch/h) TLR5-V5: 100 ng or mutated chicken (ch\*) TLR5-V5 (amino acid 744 N to A): 200 ng. 48 h post transfection, cells were coincubated with purified recombinant *Salmonella* FliC (50 ng/well), followed by a measurement of secreted IL-8 in cell supernatants. Mean and standard deviation from technical triplicates of biological duplicates of one representative experiment are shown. **(A)**. Western Blot analysis of cleared lysates of transfected HeLa cells detected using anti-V5 antibody, anti-P-p38 antibody and anti-p38 antibody **(B)** (see Table S6).



**Faber et al., Figure S6**

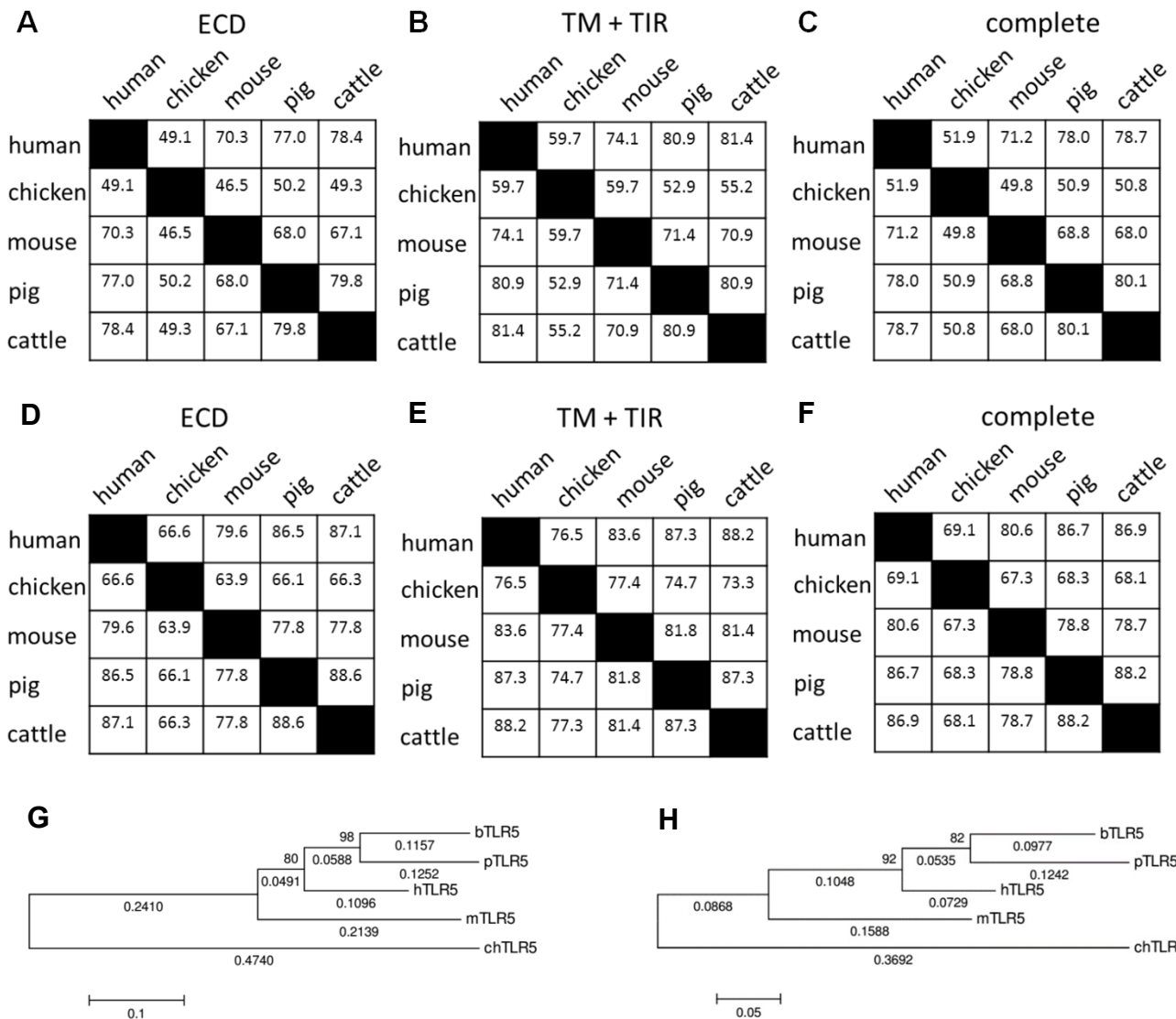
**Fig. S6: controls for experimental setup of ECD-dependent TLR5 signaling screening system. A)** Non-linear regression curves of dose responses of chimeric TLR5 receptors for determination of optimal activation doses for the system (measured original values shown in Fig. 3 B). Values are depicted relative to a reference (corresponding TLR5 construct activated by maximal dosage of FliC), set as 100%. Regression was performed using GraphPad Prism 6 using the variable slope model (four parameter dose-response curve). **B)** NF-κB activation of human TLR5 by increasing amounts of *C. jejuni* and *S. Typhimurium* lysates (dose dependency determination). HEK-Blue Null1 NF-κB reporter cells were transiently transfected with 100 ng DNA of human TLR5-V5. After 24 hours, transfected cells were coincubated with 200 ng to 3 µg protein content of *C. jejuni* (isolate Cj 73 or Cj 75) or *S. Typhimurium* whole bacterial lysates per well for 11 h; NF-κB-dependent SEAP production was determined by colorimetric measurements at 620 nm (see Methods). Absolute values of the colorimetric tests are shown. Background activation was determined by activation of empty vector-transfected cells by the corresponding lysate and subtracted from each test value to quantify specifically the TLR5-dependent activation. Mean and standard deviation from technical triplicates of a representative experiment are shown.



Faber *et al.*, Figure S7

**Fig. S7: comparison of flagellin expression by selected *Salmonella enterica* serovars (A) and *C. jejuni* (B) isolates in whole bacterial lysates.** Western Blot analysis of selected *Salmonella* or *C. jejuni* lysates (5 µg total protein/lane) using anti-*E. coli* flagellin antibody, which specifically recognizes flagellin proteins in *Salmonella* and *Campylobacter*. *S. Enteritidis* B554 only expresses FliC, while *S. Choleraesuis* B815 expresses only FliB.





**Faber et al., Figure S8**

**Fig. S8: pairwise amino acid sequence comparison of TLR5 from various vertebrate species. A to F:** matrix depiction of pairwise amino acid sequence comparison of TLR5 from different vertebrate species regarding amino acid sequence identity (in [%], **A to C**) or amino acid sequence similarity (in [%], **D to F**) using pairwise alignment and final analysis with Needle ([http://www.ebi.ac.uk/Tools/psa/emboss\\_needle/](http://www.ebi.ac.uk/Tools/psa/emboss_needle/)). ECD: ectodomain, TM: transmembrane domain, TIR: Toll/interleukin-1 receptor homology domain. ECD (**A, D**), TM plus TIR (**B, E**) and complete sequences (**C, F**) were analyzed separately. **G and H**) Maximum Likelihood Trees of ECD (**G**) and TM plus TIR (**H**) of TLR5 from different species (human (h), chicken (ch), mouse (m), pig (p) and bovine (b) TLR5) generated with MEGA5.2 (Tamura et al., 2011) including 500 bootstrap repetitions.

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          *           20           *           40           *
B554_FliC : -LNKSSSSLSSAEEFLSSGLRINSA DDAAGQAIANRFTSNINGLTQASRNANDGIS : 56
B2359_FliC : -LNKSSSSLSSAEEFLSSGLRINSA DDAAGQAIANRFTSNINGLTQASRNANDGIS : 56
B2486_FliC : -LNKSSSSLSSAEEFLSSGLRINSA DDAAGQAIANRFTSNINGLTQASRNANDGIS : 56
B2334_FliC : -LNKSSSSLSSAEEFLSSGLRINSA DDAAGQAIANRFTSNINGLTQASRNANDGIS : 56
R160_FliC : -LNKSSSALGTAEFLSSGLRINSA DDAAGQAIANRFTANINGLTQASRNANDGIS : 56
B1533_FliC : -LNKSSSALGTAEFLSSGLRINSA DDAAGQAIANRFTANINGLTQASRNANDGIS : 56
B1830_FliC : -LNKSSSAXGTAEFLSSGLRINSA DDAAGQAIANRXTANINGLTQASRNANDGIS : 56
B1040_FliC : -LNKSSSALGTAEFLSSGLRINSA DDAAGQAIANRFTANINGLTQASRNANDGIS : 56
B1085_FliC : -LNKSSSALGTAEFLSSGLRINSA DDAAGQAIANRFTANINGLTQASRNANDGIS : 56
B2075_FliC : -LNKSSSALGTAEFLSSGLRINSA DDAAGQAIANRFTANINGLTQASRNANDGIS : 56
B2078_FliC : -LNKSSSALGTAEFLSSGLRINSA DDAAGQAIANRFTANINGLTQASRNANDGIS : 56
B2183_FliC : -LNKSSSALGTAEFLSSGLRINSA DDAAGQAIANRFTANINGLTQASRNANDGIS : 56
B2257_FliC : -LNKSSSALGTAEFLSSGLRINSA DDAAGQAIANRFTANINGLTQASRNANDGIS : 56
B2396_FliC : -LNKSSSALGTAEFLSSGLRINSA DDAAGQAIANRFTANINGLTQASRNANDGIS : 56
xy82_FliC : -LNKSSSALGTAEFLSSGLRINSA DDAAGQAIANRFTANINGLTQASRNANDGIS : 56
R269_FliC : -LNKSSSALGTAEFLSSGLRINSA DDAAGQAIANRFTANINGLTQASRNANDGIS : 56
xy810_FliC : -LNKSSSALGTAEFLSSGLRINSA DDAAGQAIANRFTANINGLTQASRNANDGIS : 56
B571_FliC : ---PSALGTAEFLSSGLRINSA DDAAGQAIANRFTANINGLTQASRNANDGIS : 53
B1951_FliC : -LNKSSSALGTAEFLSSGLRINSA DDAAGQAIANRFTANINGLTQASRNANDGIS : 56
B560_FliC : -LNKSSSALGTAEFLSSGLRINSA DDAAGQAIANRFTANINGLTQASRNANDGIS : 57
B558_FliC : -LNKSSSALGTAEFLSSGLRINSA DDAAGQAIANRFTANINGLTQASRNANDGIS : 56
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B2359_FliC : IACTTEGALNEINNNLQVRVRELSVQATNGTNS SSDLKSIQDEIQQLLEIDRVSNQT : 113
B2486_FliC : IACTTEGALNEINNNLQVRVRELSVQATNGTNS SSDLKSIQDEIQQLLEIDRVSNQT : 113
B2334_FliC : IACTTEGALNEINNNLQVRVRELSVQATNGTNS SSDLKSIQDEIQQLLEIDRVSNQT : 113
R160_FliC : IACTTEGALNEINNNLQVRVRELAVSANSTNS SDDLDSIQAEITQRLNEIDRVSGQT : 113
B1533_FliC : IACTTEGALNEINNNLQVRVRELAVSANGTNS SDDLDSIQAEITQRLNEIDRVSGQT : 113
B1830_FliC : XACTTEGALNEINNNLQVRVRELAVSANGTNS SDDLDSIQAEITQRLNEIDRVSGQT : 113
B1040_FliC : IACTTEGALNEINNNLQVRVRELAVSANSTNS SDDLDSIQAEITQRLNEIDRVSGQT : 113
B1085_FliC : IACTTEGALNEINNNLQVRVRELAVSANSTNS SDDLDSIQAEITQRLNEIDRVSGQT : 113
B2075_FliC : IACTTEGALNEINNNLQVRVRELAVSANSTNS SDDLDSIQAEITQRLNEIDRVSGQT : 113
B2078_FliC : IACTTEGALNEINNNLQVRVRELAVSANSTNS SDDLDSIQAEITQRLNEIDRVSGQT : 113
B2183_FliC : IACTTEGALNEINNNLQVRVRELAVSANSTNS SDDLDSIQAEITQRLNEIDRVSGQT : 113
B2257_FliC : IACTTEGALNEINNNLQVRVRELAVSANSTNS SDDLDSIQAEITQRLNEIDRVSGQT : 113
B2396_FliC : IACTTEGALNEINNNLQVRVRELAVSANSTNS SDDLDSIQAEITQRLNEIDRVSGQT : 113
xy82_FliC : IACTTEGALNEINNNLQVRVRELAVSANSTNS SDDLDSIQAEITQRLNEIDRVSGQT : 113
R269_FliC : IACTTEGALNEINNNLQVRVRELAVSANSTNS SDDLDSIQAEITQRLNEIDRVSGQT : 113
xy810_FliC : IACTTEGALNEINNNLQVRVRELAVSANSTNS SDDLDSIQAEITQRLNEIDRVSGQT : 113
B571_FliC : IACTTEGALNEINNNLQVRVRELAVSANSTNS SDDLDSIQAEITQRLNEIDRVSGQT : 110
B1951_FliC : IACTTEGALNEINNNLQVRVRELAVSANSTNS SDDLDSIQAEITQRLNEIDRVSGQT : 113
B560_FliC : XACTTEGALNEINNNLQVRVRELAVSANSTNS SDDLDSIQAEITQRLNEIDRVSGQT : 114
B558_FliC : IACTTEGALNEINNNLQVRVRELAVSANSTNS SDDLDSIQAEITQRLNEIDRVSGQT : 113
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B2486_FliC : CFNGVWLSQDNQMKIQVGANDGETITIDLQKIDVKS LGLDGFNVVGPKEATVGD LK : 170
B2334_FliC : CFNGVWLSQDNQMKIQVGANDGETITIDLQKIDVKS LGLDGFNVVGPKEATVGD LK : 170
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B1533_FliC : CFNGVWLAQDNILTIQVGANDGETIDIDLKEISSKTLGLDKPNVQDAYTPKETAVT : 170
B1830_FliC : CXNGVWLAQDNILTIQVGANDGETIDIDLKEISSKTLGLDKLNVO DAYTPKETAVT : 170
B1040_FliC : CFNGVWLAQDNILTIQVGANDGETIDIDLKQINSQTLGLD TLNVQKQYKVSDTAAT : 170
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B2334_FliC : SSFKNVTGYDTYAAAGADKYRVDINSAGVVTDAVAPDKVYVNAANGQLTTDDAENNTA : 227
R160_FliC : RDVTTYKNGGTTLTAPNA-----AATDIALGTTGAA--CTAAVKFKDGN----- : 213
B1533_FliC : VDKTTYKNGTDPITAAQSN-----TDIQTALIGGGATG--VTGADIKFKDGGQYYLD- : 217
B1830_FliC : VDKTTYKNGTDPITAAQSN-----TDIQTALIGGGATG--VTGADIKFKDGGQYYLD- : 217
B1040_FliC : VTGYTDSATAIDKSTFAA-----SATTLGGTPAITG--DLK-FDDTTGKY----- : 213
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B2075_FliC : VTGYTDSATAIDKSTFAA-----SATTLGGTPAITG--DLK-FDDTTGKY----- : 213
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B2257_FliC : VTGYTDSATAIDKSTFAA-----SATTLGGTPAITG--DLK-FDDTTGKY----- : 213
B2396_FliC : VTGYTDSATAIDKSTFAA-----SATTLGGTPAITG--DLK-FDDTTGKY----- : 213
xy82_FliC : VTGYTDSATAIDKSTFAA-----SATTLGGTPAITG--DLK-FDDTTGKY----- : 213
R269_FliC : VTGYADTTIALDNSTFKA-----SATGLGGTDQKID--GDL-KFDDTTGKY----- : 213
xy810_FliC : ASYSDSKQNI-----VPD-----KATTAIKIGAATS--GGAGIKADISFKDQGY- : 213
B571_FliC : SVKLD-TSALTADAIKGG-----VTCATLACALK-D--GKVSNGTDYVVEV--- : 210
B1951_FliC : PSATLSTALDGAALKT-----GTGSTTDTGSIKD--GKVYYNSTSKNYVVEV- : 216
B560_FliC : QSLDLKTAGITGATLKAG-----IIGTTTETGTSVKD--GKVYYDADSKNYVVEV- : 218
B558_FliC : QSLDLKTAGITGATLKAG-----IIGTTTETGTSVKD--GKVYYDADSKNYVVEV- : 217

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B2486_FliC : VDLFKTTKSTAGTAEAKAIAAGAIKGGKGGDTFDYKGVFTTI-D--TKTGDDNGKVS : 281
B2334_FliC : VDLFKTTKSTAGTAEAKAIAAGAIKGGKGGDTFDYKGVFTTI-D--TKTGDDNGKVS : 281
R160_FliC : -----FVEVTGTT-----KDGLYEATVDAAGAVTMTANKATVTGASTVTENQI : 256
B1533_FliC : ----VKGGASAGVYKATYDETT-TKKVNIDITDKTPLATAEATAI-RGTATITHNQIA : 268
B1830_FliC : ----VKGGASAGVYKATYDETT-TKKVNIDITDKTPLATAEATAI-RGTATITHNQIA : 268
B1040_FliC : ----ADVSTGTTAKDG-----VYEVTVAADGK-VTLTGTP-TGPIIAGFPSTA : 254
B1085_FliC : ----ADVSTGTTAKDG-----VYEVTVAADGK-VTLTGTP-TGPIIAGFPSTA : 254
B2075_FliC : ----ADVSTGTTAKDG-----VYEVTVAADGK-VTLTGTP-TGPIIAGFPSTA : 254
B2078_FliC : ----ADVSTGTTAKDG-----VYEVTVAADGK-VTLTGTP-TGPIIAGFPSTA : 254
B2183_FliC : ----ADVSTGTTAKDG-----VYEVTVAADGK-VTLTGTP-TGPIIAGFPSTA : 254
B2257_FliC : ----ADVSTGTTAKDG-----VYEVTVAADGK-VTLTGTP-TGPIIAGFPSTA : 254
B2396_FliC : ----ADVSTGTTAKDG-----VYEVTVAADGK-VTLTGTP-TGPIIAGFPSTA : 254
xy82_FliC : ----ADVSTGTTAKDG-----VYEVTVAADGK-VTLTGTP-TGPIIAGFPSTA : 254
R269_FliC : ----YAKV---TVTGGTGGK---DGYEYVVDKTNGE-VTLAGGA-TSPLTGGLPATA : 258
xy810_FliC : ----YATVSGYDDAADIDK---NGTYEVTVAADTGAVTIFATRPT-VVDLPTDAKAVS : 262
B571_FliC : ----SFADATDS-----GK---NGFLKVDVNTTGAVTVPAAAA-NVVAARAVGSE : 254
B1951_FliC : ----EFTDATTQ-----TN---KGGFY-KVNVADDGAVTMTAAT-TKEATPTTGITE : 259
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B558_FliC : ----DFTDITDK-----AA---HAGFYKADVADADGNVSLATGAT-KEAKPTNAVEVE : 261

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B2359_FliC : TTINGEKVLTVAADIATGATDVNAATLQSSKNVYTSVNVGQFTFDDTK-NESAKLS : 337
B2486_FliC : TTINGEKVLTVAADIATGATDVNAATLQSSKNVYTSVNVGQFTFDDTK-NESAKLS : 337
B2334_FliC : TTINGEKVLTVAADIAIGAADVNAATLQSSKNVYTSVNVGQFTFDDTK-NESAKLS : 337
R160_FliC : VDAVTPTPVD---TVAAATALTNAAGVTGA--TGNTSLVKMSFEDKNGVTDAGYALKV : 309
B1533_FliC : EVTKEGVDTTVAQAALAAAGVTGAD-----KDNTSLVKLSFEDKNGVIDGGYAVKM : 320
B1830_FliC : EVTKEGVDTTVAQAALAAAGVTGAD-----KDNTSLVKLSFEDKNGVIDGGYAVKM : 320
B1040_FliC : TKDVKQTQQENADLTEAKAALTAAGVAA--AGTASVVKMSYTDNNGTIDGGLAV-K : 308
B1085_FliC : TKDVKQTQQENADLTEAKAALTAAGVAA--AGTASVVKMSYTDNNGTIDGGLAV-K : 308
B2075_FliC : TKDVKQTQQENADLTEAKAALTAAGVAA--AGTASVVKMSYTDNNGTIDGGLAV-K : 308
B2078_FliC : TKDVKQTQQENADLTEAKAALTAAGVAA--AGTASVVKMSYTDNNGTIDGGLAV-K : 308
B2183_FliC : TKDVKQTQQENADLTEAKAALTAAGVAA--AGTASVVKMSYTDNNGTIDGGLAV-K : 308
B2257_FliC : TKDVKQTQQENADLTEAKAALTAAGVAA--AGTASVVKMSYTDNNGTIDGGLAV-K : 308
B2396_FliC : TKDVKQTQQENADLTEAKAALTAAGVAA--AGTASVVKMSYTDNNGTIDGGLAV-K : 308
xy82_FliC : TKDVKQTQQENADLTEAKAALTAAGVAA--AGTASVVKMSYTDNNGTIDGGLAV-K : 308
R269_FliC : TEDVKNVQVAADLLEAKAALTAAGV--TGTASVVKMSYTDNNGTIDGGLAV-K : 310
xy810_FliC : KVQNDTEIA---ATNAKAALKAAGVADAEADTATLVKMSYTDNNGVIDGGFAFKT : 316
B571_FliC : VTEVQGLNTP---SSAVQDQLTAAGVSAAADAAKSEVVKMSYTDKNGTIDGGFGVKV : 308
B1951_FliC : VTQVQKPVAA---PA---AIQAQLTAHVITGADTAEMVVKMSYTDKNGTIDGGFGVKV : 311
B560_FliC : KTIDEKPLKA---SSVQDALKAAGIADVAEAAITVVKMSYTDKNGTIDGGYGIKV : 316
B558_FliC : KTIDEKPLKA---SSVQDALKAAGIADVAEAAITVVKMSYTDKNGTIDGGYGIKV : 315

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Continued on next page

\* 360 \* 380 \*  
 B554\_FliC : DLEANN-AVKGESKITVNGAEYTNANATDKITL-----AGKTMFID : 377  
 B2359\_FliC : DLEANN-AVKGESKITVNGAEYTNANATDKITL-----AGKTMFID : 377  
 B2486\_FliC : DLEANN-AVKGESKITVNGAEYTNANATDKITL-----AGKTMFID : 377  
 B2334\_FliC : DLEANN-AVKGESKITVNGAEYTNANATDKITL-----AGKTMFID : 377  
 R160\_FliC : GNDYYAADYDEKTGEIKAKTVNVTDAATATKTVGAVKFGGANGSIVVTATVDGNTYQA : 366  
 B1533\_FliC : GDDFYAATYDEKTGAI TAKTTTYTDGTVVAQTGAVKFGGANGSIVVTATDGKTYLA : 377  
 B1830\_FliC : GDDFYAATYDEKTGAI TAKTTTYTDGTVVAQTGAVKFGGANGSIVVTATDGKTYLA : 377  
 B1040\_FliC : VGDDYYSATONKDGSI SINTTKYTADDGTSKTAENKLG GADGNTVVSIGGKTYAAS : 365  
 B1085\_FliC : VGDDYYSATONKDGSI SINTTKYTADDGTSKTAENKLG GADGNTVVSIGGKTYAAS : 365  
 B2075\_FliC : VGDDYYSATONKDGSI SINTTKYTADDGTSKTAENKLG GADGNTVVSIGGKTYAAS : 365  
 B2078\_FliC : VGDDYYSATONKDGSI SINTTKYTADDGTSKTAENKLG GADGNTVVSIGGKTYAAS : 365  
 B2183\_FliC : VGDDYYSATONKDGSI SINTTKYTADDGTSKTAENKLG GADGNTVVSIGGKTYAAS : 365  
 B2257\_FliC : VGDDYYSATONKDGSI SINTTKYTADDGTSKTAENKLG GADGNTVVSIGGKTYAAS : 365  
 B2396\_FliC : VGDDYYSATONKDGSI SINTTKYTADDGTSKTAENKLG GADGNTVVSIGGKTYAAS : 365  
 xy82\_FliC : VGDDYYSATONKDGSI SINTTKYTADDGTSKTAENKLG GADGNTVVSIGGKTYAAS : 365  
 R269\_FliC : VGDDYYSATONKDGSI SINTTKYTADDGTSKTAENKLG GADGNTVVSIGGKTYAAS : 367  
 xy810\_FliC : SGGYYAASVDKSGAASLKVTSYVDATTGTEKTAENKLG GADGNTVVTIDGKTYNAS : 373  
 B571\_FliC : GDDIYA-ATKNKDGSI SINATTEYTDKDNNTKTAENQLGGVDGNTVVTIDGKTYNAS : 364  
 B1951\_FliC : GADIYA-ATKNKDGSI SINATTEYTDKDNNTKTAENQLGGADGNTVVSIDGKTYNAS : 367  
 B560\_FliC : GDDYYA-ATKEKDGSI SINSTSYTDKDNNTKTAENQLGGADGNTVVSIDGKTYNAS : 372  
 B558\_FliC : GDDYYA-ATKEKDGSI SINSTSYTDKDNNTKTAENQLGGADGNTVVSIDGKTYNAS : 371

G T

\* 420 \* 440 \*  
 B554\_FliC : KTA-SGVSTLINEDAAAANKKSTANFLASDSALS KVDVAVSSLAQGNFSDSAITNL : 433  
 B2359\_FliC : KTA-SGVSTLINEDAAAANKKSTANFLASDSALS KVDVAVSSLAQGNFSDSAITNL : 433  
 B2486\_FliC : KTA-SGVSTLINEDAAAANKKSTANFLASDSALS KVDVAVSSLAQGNFSDSAITNL : 433  
 B2334\_FliC : KTA-SGVSTLINEDAAAANKKSTANFLASDSALS KVDVAVSSLAQGNFSDSAITNL : 433  
 R160\_FliC : SDVKGHNFSQSGALSEAVTTKTENFLAKDAALAQVDALRSDLAGVGNFNSAITNL : 423  
 B1533\_FliC : SDLDKHNFRGTGELKEVNTDKTENFLQKDAALAQVDTLRSDLAGVGNFNSAITNL : 434  
 B1830\_FliC : SDLDKHNFRGTGELKEVNTDKTENFLQKDAALAQVDTLRSDLAGVGNFNSAITNL : 434  
 B1040\_FliC : KAE-GHNFKAQPDLAEEAATTTENFLQKDAALAQVDTLRSDLAGVGNFNSAITNL : 421  
 B1085\_FliC : KAE-GHNFKAQPDLAEEAATTTENFLQKDAALAQVDTLRSDLAGVGNFNSAITNL : 421  
 B2075\_FliC : KAE-GHNFKAQPDLAEEAATTTENFLQKDAALAQVDTLRSDLAGVGNFNSAITNL : 421  
 B2078\_FliC : KAE-GHNFKAQPDLAEEAATTTENFLQKDAALAQVDTLRSDLAGVGNFNSAITNL : 421  
 B2183\_FliC : KAE-GHNFKAQPDLAEEAATTTENFLQKDAALAQVDTLRSDLAGVGNFNSAITNL : 421  
 B2257\_FliC : KAE-GHNFKAQPDLAEEAATTTENFLQKDAALAQVDTLRSDLAGVGNFNSAITNL : 421  
 B2396\_FliC : KAE-GHNFKAQPDLAEEAATTTENFLQKDAALAQVDTLRSDLAGVGNFNSAITNL : 421  
 xy82\_FliC : KAE-GHNFKAQPDLAEEAATTTENFLQKDAALAQVDTLRSDLAGVGNFNSAITNL : 421  
 R269\_FliC : KAE-GHNFKAQPDLAEEAATTTENFLQKDAALAQVDTLRSDLAGVGNFNSAITNL : 423  
 xy810\_FliC : KAA-GHNFKAQPELAEAAATTTENFLQKDAALAQVDALRSDLAGVGNFNSAITNL : 429  
 B571\_FliC : KAA-GHDFKAQPELAEAAATTTENFLQKDAALAQVDALRSDLAGVGNFNSAITNL : 420  
 B1951\_FliC : KAA-GHNFKAQPELAEAAATTTENFLQKDAALAQVDALRSDLAGVGNFNSAITNL : 423  
 B560\_FliC : KAA-GHNFKAQPDLAEEAATTTENFLQKDAALAQVDALRSDLAGVGNFNSAITNL : 428  
 B558\_FliC : KAA-GHNFKAQPDLAEEAATTTENFLQKDAALAQVDALRSDLAGVGNFNSAITNL : 427

T NPL ID AL VD 6RS LGA6QNR F1SAITNL

460 \* 480 \* 500 \*  
 B554\_FliC : GNTVTNLSAARSRIEDADYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 488  
 B2359\_FliC : GNTVTNLSAARSRIEDADYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 488  
 B2486\_FliC : GNTVTNLSAARSRIEDADYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 488  
 B2334\_FliC : GNTVTNLSAARSRIEDADYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 488  
 R160\_FliC : GNTVNNLSEAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 478  
 B1533\_FliC : GNTVNNLSSAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 489  
 B1830\_FliC : GNTVNNLSSAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 489  
 B1040\_FliC : GNTVNNLSSAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 476  
 B1085\_FliC : GNTVNNLSSAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 476  
 B2075\_FliC : GNTVNNLSSAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 476  
 B2078\_FliC : GNTVNNLSSAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 476  
 B2183\_FliC : GNTVNNLSSAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 476  
 B2257\_FliC : GNTVNNLSSAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 476  
 B2396\_FliC : GNTVNNLSSAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 476  
 xy82\_FliC : GNTVNNLSSAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 476  
 R269\_FliC : GNTVNNLSSAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 478  
 xy810\_FliC : GNTVNNLSSAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 484  
 B571\_FliC : GNTVNNLSSAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 475  
 B1951\_FliC : GNTVNNLSSAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 478  
 B560\_FliC : GNTVNNLSSAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 483  
 B558\_FliC : GNTVNNLSSAARSRIEDSDYATEVSNMSPAQILOCA TSVLAQANQVPQNVLSLLR\* : 482

GNTV NL ARSRIED DYATEVSNMSPAQILOQAGTSVLAQANQVPQNVLSLLR

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## Faber *et al.*, Figure S9

**Fig. S9: amino acid alignment of FliC flagellin sequences of *Salmonella* isolates used in this study.** *fliC* genes of *Salmonella* isolates were amplified using the primers FSa1 and rFSa1 (Dauga *et al.*, 1998) and Sanger-sequenced using the same primers and additionally primer rFSa1\_2 (see Suppl. Tab. 7). Derived amino acid sequences (first 17 amino acids not sequenced) were aligned using ClustalOmega (Sievers *et al.*, 2011) and depicted according to their physico-chemical properties using GeneDoc (Nicholas *et al.*, 1997). Strain numbers of strains listed in Table 1 which are included in this alignment but not in Fig. S10 only possess the *fliC* gene (e.g. B571) and served as experimental controls for the strains' FliC flagellin-specific activation potential.



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*      240      *      260      *      280      *      300
B558_F1jB : A-DTANNGKYEVTVDSATFAVSFGATPTSTVT-SDTAVTK--VQVNAFVAADAATKALQDGGVSSADASAATLV : 297
B560_F1jB : A-DTANNGKYEVTVDSATFAVSFGATPTSTVT-SDTAVTK--VQVNAFVAADAATKALQDGGVSSADASAATLV : 297
xy82_F1jB : A-DAAANGDYEVNVATD-KVTLATGATTTMPAGAATKTEVLELKDTFAVVSADANALIAGGVDTADANAATLV : 301
R269_F1jB : A-DAAANGDYEVNVATD-STVTLAAGATTTMPAGATTKTEVLELKDTFAVVSADANALIAGGVDTADANGAELV : 301
R160_F1jB : A-DAAANGDYEVNVATD-STVTLAAGATTTMPASATTKTEVLELKDTFAVVSADANALIAGGVDTADANGAELV : 302
xy810_F1jB : A-DAAANGDYEVNVATD-STVTLAAGATTTMPAGATTKTEVLELKDTFAVVSADANALIAGGVDTADANGAELV : 302
B815_F1jB : A-DAAANGDYEVNVATD-STVTLAAGATTTMPAGATTKTEVLELKDTFAVVSADANALIAGGVDTADANGAELV : 301
B1951_F1jB : A-DAAANGDYEVNVATD-KVTLATSATTTMPAGAATKTEVLELKDTFAVVSADANALIAGGVDTADANAATLV : 301
B1040_F1jB : A-DAAANGDYEVNVATD-STVTLAAGATTTMPAGVTTKTEVLELTTFVVSADANALIAGGVDTADANAATLV : 301
B1085_F1jB : A-DAAANGDYEVNVATD-STVTLAAGATTTMPAGVTTKTEVLELTTFVVSADANALIAGGVDTADANAATLV : 301
B2078_F1jB : A-DAAANGDYEVNVATD-STVTLAAGATTTMPAGVTTKTEVLELTTFVVSADANALIAGGVDTADANAATLV : 301
B2183_F1jB : A-DAAANGDYEVNVATD-STVTLAAGATTTMPAGVTTKTEVLELTTFVVSADANALIAGGVDTADANAATLV : 301
B2257_F1jB : A-DAAANGDYEVNVATD-STVTLAAGATTTMPAGVTTKTEVLELTTFVVSADANALIAGGVDTADANAATLV : 301
B2396_F1jB : A-DAAANGDYEVNVATD-STVTLAAGATTTMPAGVTTKTEVLELTTFVVSADANALIAGGVDTADANAATLV : 301
B1050_F1jB : A-DAAANGDYEVNVATD-STVTLAAGATTTMPAGVTTKTEVLELTTFVVSADANALIAGGVDTADANAATLV : 301
xy92_F1jB : A-DAAANGDYEVNVATD-STVTLAAGATTTMPAGVTTKTEVLELTTFVVSADANALIAGGVDTADANAATLV : 301
2075_F1jB : A-DAAANGDYEVNVATD-STVTLAAGATTTMPAGVTTKTEVLELTTFVVSADANALIAGGVDTADANAATLV : 301
A DaAKNGdYEVnVa3d G V3laagaTK3T6paG ttkTevqel tP v sAdaKNaLiaGGVd DAN A LV

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*      320      *      340      *      360      *      380
B558_F1jB : MSYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 373
B560_F1jB : MSYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 373
xy82_F1jB : MXYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 377
R269_F1jB : MSYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 377
R160_F1jB : MSYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 378
xy810_F1jB : MSYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 378
B815_F1jB : MSYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 377
B1951_F1jB : MSYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 377
B1040_F1jB : MSYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 377
B1085_F1jB : MSYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 377
B2078_F1jB : MSYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 377
B2183_F1jB : MSYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 377
B2257_F1jB : MSYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 377
B2396_F1jB : MSYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 377
B1050_F1jB : MSYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 377
xy92_F1jB : MSYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 377
2075_F1jB : MSYTDNNGKTIIEGGYALRAGDYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKT YNA : 377
KMsYTDKNGKTIIEGGYALKAGDKYYAADYDEATGAIKAKTTSYTAADGTTKTAANQLGGVDGKTEVVTIDGKTYNA

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*      400      *      420      *      440      *
B558_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 449
B560_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 449
xy82_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 453
R269_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 453
R160_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 454
xy810_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 454
B815_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 453
B1951_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 453
B1040_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 453
B1085_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 453
B2078_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 453
B2183_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 453
B2257_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 453
B2396_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 453
B1050_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 453
xy92_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 453
2075_F1jB : SAAAGDFKACPELAAAAAATTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY : 453
SKAAGHDFKACPELAAEAANKTTENPLQKIDAALAQVDALRSDLGAVQNRFNSAITNLGNTVNNLSAASRIEDSDY

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Continued on next page

	460	*	480	*																																			
B558_F1jB	:	A	T	V	S	N	M	S	A	I	L	C	A	G	T	S	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	485						
B560_F1jB	:	A	T	V	S	N	M	S	A	I	L	C	A	G	T	S	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	485						
xy82_F1jB	:	A	T	V	S	N	M	S	A	I	L	C	A	G	T	S	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	489						
R269_F1jB	:	A	T	V	S	N	M	S	A	I	L	C	A	G	T	S	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	489						
R160_F1jB	:	A	T	V	S	N	M	S	A	I	L	C	A	G	T	S	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	490						
xy810_F1jB	:	A	T	V	S	N	M	S	A	I	L	C	A	G	T	S	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	490						
B815_F1jB	:	A	T	V	S	N	M	S	A	I	L	C	A	G	T	S	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	489						
B1951_F1jB	:	A	T	V	S	N	M	S	A	I	L	C	A	G	T	S	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	489						
B1040_F1jB	:	A	T	V	S	N	M	S	A	I	L	C	A	G	T	S	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	489						
B1085_F1jB	:	A	T	V	S	N	M	S	A	I	L	C	A	G	T	S	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	489						
B2078_F1jB	:	A	T	V	S	N	M	S	A	I	L	C	A	G	T	S	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	489						
B2183_F1jB	:	A	T	V	S	N	M	S	A	I	L	C	A	G	T	S	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	489						
B2257_F1jB	:	A	T	V	S	N	M	S	A	I	L	C	A	G	T	S	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	489						
B2396_F1jB	:	A	T	V	S	N	M	S	A	I	L	C	A	G	T	S	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	489						
B1050_F1jB	:	A	T	V	S	N	M	S	A	I	L	C	A	G	T	S	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	489						
xy92_F1jB	:	A	T	V	S	N	X	S	A	I	L	C	A	G	T	X	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	489						
2075_F1jB	:	A	T	V	S	N	M	S	A	I	L	C	A	G	T	S	V	L	A	A	N	V	P	N	V	L	S	L	L	R	*	:	489						
		A	T	E	V	S	N	m	S	R	A	Q	I	L	Q	Q	A	G	T	s	V	L	A	Q	A	N	Q	V	P	Q	N	V	L	S	L	L	R		

**Faber et al., Figure S10**

**Fig. S10: amino acid alignment of FljB flagellin sequences of *Salmonella* isolates used in this study.** *fljB* genes of *Salmonella* isolates were amplified using the primers FSa2 and rFSa2 (Dauga et al., 1998) and Sanger-sequenced using the same primers and additionally primer rFSa1\_3 (see Suppl. Tab 7). Derived amino acid sequences (first 16 or 17 amino acids not sequenced) were aligned with ClustalOmega (Sievers et al., 2011) and depicted according to their physico-chemical properties using GeneDoc (Nicholas et al., 1997). Strain numbers of strains listed in Table 1 which are included in this alignment but not in Fig. S9 only possess the *flgJ* gene (e.g. B815) and served as controls for the strains' FlgJ flagellin-specific activation potential.





chTLR5	flsvtllmflmstiiiftrcrgicfvwyktttktligshppaadtsseymydaylcyskndf	706
mTLR5	lctvtltlflvitlvvikfrgicfcicyktaqrlvfkdkvwslepgayrydaylcfsskdf	719
hTLR5	vctvtltlflmtilvtvkfrgfcfcicyktaqrlvfkdhpggqtepdmykydaylcfsskdf	704
bTLR5	lvtvtltlflvitlavtkfrgfcfcicykkaqsllfkdpikgresdtykydaylcfsskdf	704
pTLR5	lftvtltlflfvailvmmkfrgfcfcicykqvrlvfkdpislgresdtykydaylcfsgkdf	703
	. :*** :::: : . : **:**: **. : : . : . * ****:** * :**	
chTLR5	ewvqnsllkhldsqqyfdknrftlcfceerdfpgeehinnirdaiwksrkticvvtqrqflk	766
mTLR5	ewaqnallkhldahyssrnrllrlcfceerdfipgenhisniqaavwgsrktvclvsrhflr	779
hTLR5	twvqnallkhldtqysdqnrfnlcfceerdfvpgenrianiqdaiwnsrkivclvsrhflr	764
bTLR5	ewvqnall-hldvqyhsqnrfnlcfceerdfmpgenhianiqdavwssrkivclvsrhflr	763
pTLR5	ewvqeallknldaqysdqnrlnlcfceerdfvpgedrisniqdavwssrkvvclvsrhflr	763
	*. :*** :** * :. :** : *****:**:** * ** : * * ** * :* :* :** :	
chTLR5	dgwcvcafnfaqsryfsdlkevlmrvvgsqyqlmkhkpriiflqrsrylrwpedyqd	826
mTLR5	dgwcleafryaqsrsldksilivvvvgsqyqlmrhetirgflqkqqylrwpedlqd	839
hTLR5	dgwcleafsyaggrclsdlnsalimvvvgsqyqlmkhqsirgfvqkqqylrwpedfkd	824
bTLR5	dgwcleafsyagsrcladlmgalimvvvgsqfhlmrhqsirgfvqkrqylrwpedlqd	823
pTLR5	dgwcleafsyagsrcladlmgtlmrvvgsqyqlmkhqsirgfvkrqyylrwpedlqd	823
	***:** * :** * :. :** : ** :***** ** :*** : ** * : : ***** **	
chTLR5	igwfldnlssqilkekkvqrvnsvgielqtiatvsh	861
mTLR5	vgwfldklsqgilkeekakkrsssigrtiatis-	873
hTLR5	vgwflhklssqilkekekkkdnniplqtvatis-	858
bTLR5	vdwflnklsqgilkekerkkdsaiqlqnvttis-	857
pTLR5	vswflnklsqyilkvkekkkdsdiqlqsv-tis-	856
	:.***:** * :** : * :. . * * :. : :	

## Faber *et al.*, Figure S11

**Fig. S11: amino acid alignment of all full-length TLR5 proteins expressed from pEF6-V5 constructs and used for activation in this study.** chTLR5 = *Gallus gallus* TLR5; m = *Mus musculus* TLR5; hTLR5 = *Homo sapiens* TLR5; bTLR5 = *Bos taurus* TLR5; pTLR5 = *Sus scrofa* TLR5. Symbols below the alignment designate the level of amino acid (aa) identity or conservation at each site: asterisk = identical aa; two dots = highly conserved aa; one dot = moderately conserved aa. Nucleotide sequences of each cloned insert were determined by Sanger sequencing and translated into amino acid sequences. The alignment was prepared using ClustalOmega (Sievers *et al.*, 2011).

## Supplementary Tables

**Table S1:** *Campylobacter jejuni* or *Salmonella enterica* isolates used in this study.

Bacterial isolate	Isolate designation		Isolated from	Host species
<i>Campylobacter jejuni</i> (ST-50)	68		Human feces	Humans, poultry, swine, cattle, sheep, cats, dogs
<i>Campylobacter jejuni</i> (ST-45)	69		Human feces	s. a.
<i>Campylobacter jejuni</i> (ST-50)	70		Human feces	s. a.
<i>Campylobacter jejuni</i> (ST-21)	71		Human feces	s. a.
<i>Campylobacter jejuni</i> (ST-50)	72		Human feces	s. a.
<i>Campylobacter jejuni</i> (ST-21)	73		Human feces	s. a.
<i>Campylobacter jejuni</i> (ST-45)	75		Human feces	s. a.
<i>Campylobacter jejuni</i> (ST-21)	87		Human feces	s. a.
<i>Campylobacter jejuni</i> (ST-21)	89		Human feces	s. a.
<i>Campylobacter jejuni</i> (ST-21)	90		Human feces	s. a.
<i>Campylobacter jejuni</i> ATCC 700819	NCTC 11168		Human feces	s. a.
<i>Campylobacter jejuni</i> (ST-21)	FBI 04321 <sup>A</sup>		Aviary bird	s. a.
<i>Campylobacter jejuni</i> (ST-21)	FBI 04371 <sup>A</sup>		Sheep	s. a.
<i>Campylobacter jejuni</i> (ST-21)	1		Broiler	s. a.
<i>Campylobacter jejuni</i> (ST-21)	FBI 04197 <sup>A</sup>		Cattle feces	s. a.
<i>Campylobacter jejuni</i> (ST-21)	FBI 02626 <sup>A</sup>		Broiler	s. a.
<i>Campylobacter jejuni</i> (ST-21)	FBI 04199 <sup>A</sup>		Cattle feces	s. a.
<i>Salmonella enterica</i> serovar Bovismorbificans	xy	82	n. s.	Cattle, horses
<i>Salmonella enterica</i> serovar Choleraesuis	B	815	Human blood	Swine
<i>Salmonella enterica</i> serovar Choleraesuis	xy	810	n. s.	s. a.
<i>Salmonella enterica</i> serovar Typhimurium	RV	269	n. s.	Humans, cattle, swine, sheep, horses, rodents, poultry
<i>Salmonella enterica</i> serovar Enteritidis	B	554	Human feces	Humans, rodents, poultry, horses
<i>Salmonella enterica</i> serovar Enteritidis	B	558	Human feces	s. a.
<i>Salmonella enterica</i> serovar Enteritidis	B	560	Human feces	s. a.
<i>Salmonella enterica</i> serovar Enteritidis	B	571	Human feces	s. a.
<i>Salmonella enterica</i> serovar Enteritidis	B	2334	Human blood	s. a.
<i>Salmonella enterica</i> serovar Enteritidis	B	2359	Human feces	s. a.
<i>Salmonella enterica</i> serovar Enteritidis	B	2486	Human feces	s. a.
<i>Salmonella enterica</i> serovar Infantis	B	1040	Human urine	Humans, poultry
<i>Salmonella enterica</i> serovar Infantis	B	1050	Human feces	s. a.
<i>Salmonella enterica</i> serovar Infantis	B	1085	Human feces	s. a.
<i>Salmonella enterica</i> serovar Infantis	B	2075	Human feces	s. a.
<i>Salmonella enterica</i> serovar Infantis	B	2078	Human feces	s. a.
<i>Salmonella enterica</i> serovar Infantis	B	2183	n. s.	s. a.
<i>Salmonella enterica</i> serovar Infantis	B	2257	n. s.	s. a.
<i>Salmonella enterica</i> serovar Infantis	B	2396	Human feces	s. a.
<i>Salmonella enterica</i> serovar Infantis	xy	92	n. s.	s. a.
<i>Salmonella enterica</i> serovar Paratyphi A	B	1951	Human blood	Humans
<i>Salmonella enterica</i> serovar Paratyphi B	RV	160	n. s.	Humans

<i>Salmonella enterica</i> serovar Typhi	B	1533	Human feces	Humans
<i>Salmonella enterica</i> serovar Typhi	B	1830	Human blood	s. a.

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ST: sequence type

n. s.: not specified

s. a.: see above

<sup>A</sup>: *C. jejuni* isolates published in (Gripp *et al.*, 2011)

**Table S2:** Plasmids used in this study.

Plasmid	Vector	Description	Resistance	Reference or Source
	pEF6-V5	mammalian protein expression vector, originated from pEF6-TLR5-V5 by excision of the insert and removal of His-tag, EF-1 $\alpha$ promoter, V5-tag	Amp <sup>R</sup>	(Lee <i>et al.</i> , 2003)
pEF6-hTLR5	pEF6-V5	protein expression plasmid for human TLR5; removal of His-tag	Amp <sup>R</sup>	(Smith, Jr. <i>et al.</i> , 2003; Lee <i>et al.</i> , 2003)
pCJ800	pEF6-V5	protein expression plasmid for mouse TLR5 (pEF6-mTLR5-V5)	Amp <sup>R</sup>	This study
pCJ801	pEF6-V5	protein expression plasmid for chicken TLR5 (pEF6-chTLR5-V5)	Amp <sup>R</sup>	This study
pCJ1413	pEF6	Protein expression plasmid for chTLR5 without V5	Amp <sup>R</sup>	This study
pCJ1414	pEF6-V5	protein expression plasmid for pig TLR5 (pEF6-piTLR5-V5)	Amp <sup>R</sup>	This study
pCJ1406	pEF6-V5	protein expression plasmid for bovine TLR5 (pEF6-boTLR5-V5)	Amp <sup>R</sup>	This study
pCJ1045	pEF6-V5	protein expression plasmid for chimeric mouse/human TLR5 (pEF6-m/hTLR5-V5)	Amp <sup>R</sup>	This study
pCJ1040	pEF6-V5	protein expression plasmid for chimeric chicken/human TLR5 (pEF6-ch/hTLR5-V5)	Amp <sup>R</sup>	This study
pCJ1044	pEF6-V5	protein expression plasmid for chimeric pig/human TLR5 (pEF6-pi/hTLR5-V5)	Amp <sup>R</sup>	This study
pCJ1407	pEF6-V5	protein expression plasmid for chimeric bovine/human TLR5 (pEF6-bo/hTLR5-V5)	Amp <sup>R</sup>	This study
	pET28a	Protein expression vector, T7 <i>lac</i> promoter, 6xHis tag, T7 tag	Km <sup>R</sup>	Novagen
pCJ375	pET28a	<i>S. enterica</i> serovar Typhimurium LT2 <i>fliC</i> cloned into pET28a via BamHI and XhoI	Km <sup>R</sup>	S. K. Lee and C. Josenhans, unpublished

Amp<sup>R</sup>: ampicillin resistance, Km<sup>R</sup>: kanamycin resistance

**Table S3:** Oligonucleotides used for gene amplification and cloning.

Gene	Primers	Sequence (5'-3') <sup>a</sup>	T <sub>m</sub> (°C)	Reference
<i>SalTy</i> LT2 <i>fliC</i>	SalLT2FliCBamHI(F)	<u>CGGGATCC</u> ATGGCACAAAGTCAT TAATAC	49	S. K. Lee and C. Josenhans, unpublished
	SalT2FliCEcoRI(R)	CGGAATT <u>CCGCAGTAAAGAGAG</u> GACG	51	S. K. Lee and C. Josenhans, unpublished
<i>chTLR5</i>	chTLR5_F1	AAAGGTACCGAGTCCGGATCCA TGATGTTACA ATCAACGGCTAATAATTG	65	This study
	chTLR5_R1	AAAAGCGGCC <u>CGCGTGTGAGA</u> CTGTCGCTATA GTTTG	69	This study
	chTLR5_R2	<u>AGA</u> ACTTAAGTGTCTGCTGGAG	55	This study
<i>mTLR5</i>	mTLR5_F2	AAAAGGTACCGCCGCCACCATG GCATGTCAACTTGACTTG	65	This study
	mTLR5_R2	AAAAGGTACCGCCGCCACCATG GCATGTCAACTTGACTTG	51	This study
	mTLR5_R4	GGAAA <u>ACTTAAAGAGACCC</u> ATG GCTTC	57	This study
<i>piTLR5</i>	piTLR5_F1	AAAAGGTACCGAGTCCGGATCC ATGGGAGACTGCCTGGTCCTG	59	This study
	piTLR5_F2	<u>ACCAAGTACATGGGAGTCCGGA</u> TCCATGGGAGACTGCCTGGTC	53	This study
	piTLR5_R1	AAAAGCGGCC <u>CGCGGAGATGG</u> TCACGCTTTGCAAC	67	This study
	piTLR5_R2	AAA <u>ACTTAAGGGACTCTAAGAC</u>	55	This study
<i>boTLR5</i>	boTLR5_BstXI_F	AAACCAAGTACATGGGCCGCCA CCATGGGAGACTGCCTTGA	51	This study
	boTLR5_NotI_R2	AAAGCGGCC <u>CGCGGAGATGGT</u> GGTTACATTTT	51	This study
	boTLR5_BsaI_R	GGAAA <u>ACTTAAAGAGACCTAAA</u> ACTTC	51	This study
<i>hMyD88</i>	hMyD88_F1	TAAGAAGGACCAGCAGAGCC	59	S. K. Lee and C. Josenhans, unpublished
	hMyD88_R1	CATGTAGTCCAGCAACAGCC	59	S. K. Lee and C. Josenhans, unpublished

<sup>a</sup>restriction sites in oligonucleotides are underlined; T<sub>m</sub>: annealing temperature.

**Table S4:** Oligonucleotides used for sequencing.

<b>Gene</b>	<b>Primers</b>	<b>Sequence (5'-3')<sup>a</sup></b>	<b>T<sub>m</sub> (°C)</b>	<b>Reference</b>
	pEF6_R2	ACAGATGGCTGGCAACTAGAAG	61	C. Josenhans, unpublished
<i>hTLR5</i>	hTLR5_2025_R	CTGGGCTGTCTTATAACAG	56	This study
<i>chTLR5</i>	chTLR5_F1	AAAGGTACCGAGTCCGGATCCATG ATGTTACATCAACGGCTAATAATTG	65	This study
	chTLR5_R2	AGAACTTAAGTGTCTGCTGGAG	55	This study
<i>piTLR5</i>	piTLR5_F1	AAAAGGTACCGAGTCCGGATCCAT GGGAGACTGCCTGGTCCTG	59	This study
	piTLR5_R2	AAAACCTTAAGGGACTCTAAGAC	55	This study
<i>boTLR5</i>	boTLR5_738_F	AGCAATGCCATCAATGGGAG	55	This study
	boTLR5_1438_F	TTGGAGAAAATATGTTGCAGC	53	This study
	pUNO1_hEF1-HTLV_F	TCCCTTGGAGCCTACCTA	51	This study
	pUNO1_SV40_R	ATTGCAGCTTATAATGGTTAC	51	This study
<i>Sal_fliC</i>	FSa1	CAAGTCATTAATACMAACAGCC	55	(Dauga <i>et al.</i> , 1998)
	rFSa1	TTAACGCAGTAAAGAGAGGAC	55	(Dauga <i>et al.</i> , 1998)
	rFSa1_2	CGTAGCCGTATCGAAGAT	49	This study
<i>Sal_fliB</i>	rFSa2	CAAGTAATCAACACTAACAGTC	58	(Dauga <i>et al.</i> , 1998)
	rFSa2	TTAACGTAACAGAGACAGCAC	58	(Dauga <i>et al.</i> , 1998)
	rFSa1_3	CAACCTGTCTGAAGCGCGTAG	61	This study

**Table S5:** Oligonucleotides used for site-directed mutagenesis.

<b>Gene</b>	<b>Primers</b>	<b>Sequence (5'-3')<sup>a</sup></b>	<b>T<sub>m</sub> (°C)</b>	<b>Reference</b>
Insertion of stop codon upstream of V5 tag	pEF6_noV5_F	CCGCTCGAGTCTATAGGGCCCGC GGTTCG	>81	This study
	pEF6_noV5_R	CGAACCGCGGGCCCTATAGACTC GAGCGG	>81	This study
Change N to A in chTLR5 (aa 744)	chTLR5_Ala_F	GCCTGGGGAAGAACATATCGCCA ATATTCGTGATGCTATTTGG	>81	This study
	chTLR5_Ala_R	CCAAATAGCATCACGAATATTGGC GATATGTTCTTCCCCAGGC	>81	This study

aa: amino acid



**Table S6:** Antibodies used in this study.

Antibody <sup>a</sup>	Application	Description/Dilution	Source
$\alpha$ -V5	WB, IF	Mouse, monoclonal/ 1:5,000 (WB), 1:500 (IF)	Invitrogen, R960-25
$\alpha$ -actin <sup>a</sup>	WB	Mouse, monoclonal/1:30,000	Chemicon, MAB1501
$\alpha$ -GAPDH <sup>a</sup>	WB	Rabbit, monoclonal/1:1,000	Cell Signaling, 14C10
$\alpha$ -p38 <sup>a</sup>	WB	Rabbit, polyclonal/1:1,000	Cell Signaling, 9212
$\alpha$ -P-p38 <sup>a</sup> (Thr180/Tyr182)	WB	Rabbit, polyclonal/1:1,000	Cell Signaling, 9211
$\alpha$ -IRAK-I <sup>a</sup>	WB	Rabbit, polyclonal/1:500	Cell Signaling, 4359
$\alpha$ -IRAK-M <sup>a</sup>	WB	Rabbit 1:1,000	Abcam, ab16534
$\alpha$ -MyD88 <sup>a</sup>	WB	Rabbit, polyclonal/1:1,000	Abcam, ab2068
$\alpha$ - <i>E. coli</i> flagellin	WB	Mouse, monoclonal/1:500	Hölzel Diagnostika, 15D8
Goat $\alpha$ -rabbit IgG (H+L)	WB	Peroxidase-conjugated, AffiniPure/1:10,000	Dianova/Jackson
Goat $\alpha$ -mouse IgG (H+L)	WB	Peroxidase-conjugated, AffiniPure F(ab') <sub>2</sub> /1:10,000	Dianova/Jackson
Goat $\alpha$ -mouse Alexa Fluor™ 488	IF	1:5,000	Molecular Probes/ ThermoScientific

<sup>a</sup> detected species: human

WB: Western Blot

IF: Immunofluorescence

**Table S7:** Oligonucleotides used for RT-PCR of chicken (*Gallus gallus*) genes.

Gene	Primers	Sequence (5'-3') <sup>a</sup>	T <sub>m</sub> (°C)	Reference <sup>a</sup>
<i>chGAPDH</i>	chGAPDH_F2	AGGGTGGTGCTAAGCGTGTT	59	(Faber <i>et al.</i> , 2016)
	chGAPDH_R2	AAGGGTGCCAGGCAGTTG	59	(Faber <i>et al.</i> , 2016)
<i>chIL-CXCLi2</i> ( <i>chIL-8</i> )	chIL-8_F1	CTGGCCCTCCTCCTGGTTTC	59	(Faber <i>et al.</i> , 2016)
	chIL-8_R1	TGGCGTCAGCTTCACATCTTG	59	(Faber <i>et al.</i> , 2016)
<i>chIL-1β</i>	chIL-1_F1	CTGAGTCATGCATCGTTTATGTTT C	59	(Faber <i>et al.</i> , 2016)
	chIL-1_R1	AAATACCTCCACCCCGACAAG	59	(Faber <i>et al.</i> , 2016)

<sup>a</sup> primers were designed and generously provided by Bernd Kaspers (LMU Munich)

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