Figure 1. No difference in leptospiral loads and in inflammatory mediators between WT and TLR5ko mice during acute phase of leptospirosis

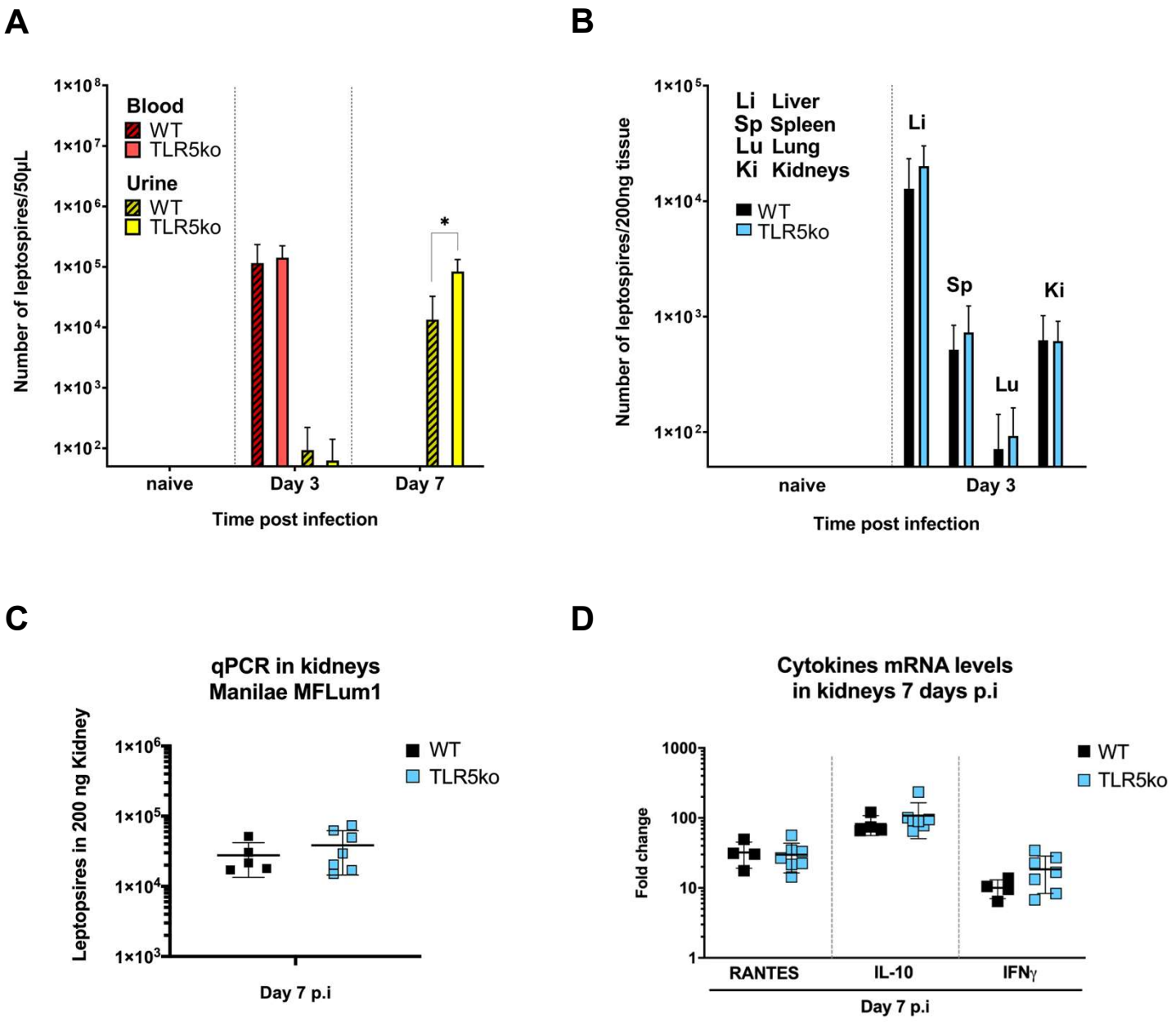


Figure 1. No difference in leptospiral loads and inflammatory mediators between WT and TLR5ko mice during acute phase of leptospirosis

A, B) Bacterial loads determined by qPCR of leptospiral DNA at several days post intraperitoneal infection of 7-week old female C57BL/6J (WT) mice and TLR5ko mice with 10^7 *L. interrogans* Manilae L495 derivative strain MFLum1; **A)** in blood (red) and urine (yellow) in WT (n= 4, hatched bars) and TLR5ko mice (n= 4, empty bars) and **B)** in liver (Li), spleen (Sp), lungs (Lu) and kidneys (Ki) from WT (n= 4, black bars) and TLR5ko (n= 4, blue bars) at day 3 post-infection (p.i). Data are expressed as mean (+/- SD) of all mice. Statistically significant differences between genotypes (Student t-test) are indicated. **C)** Bacterial loads in kidneys determined by qPCR 7 days p.i of WT mice (n= 5, black dots) and TLR5ko mice (n= 7, blue dots). **D)** Inflammation measured in kidney by mRNA expression of cytokines (RANTES, IL10, IFNγ) measured by RT-qPCR at 7 days p.i in WT mice (n= 5, black dots) and TLR5ko mice (n= 7, blue dots). Individual mice are represented as dots and lines correspond to mean (+/- SD) of all mice. No statistical difference was found between WT and TLR5ko mice.

Figure 2. No difference in kidney colonization in WT and TLR5ko mice 15 days post infection

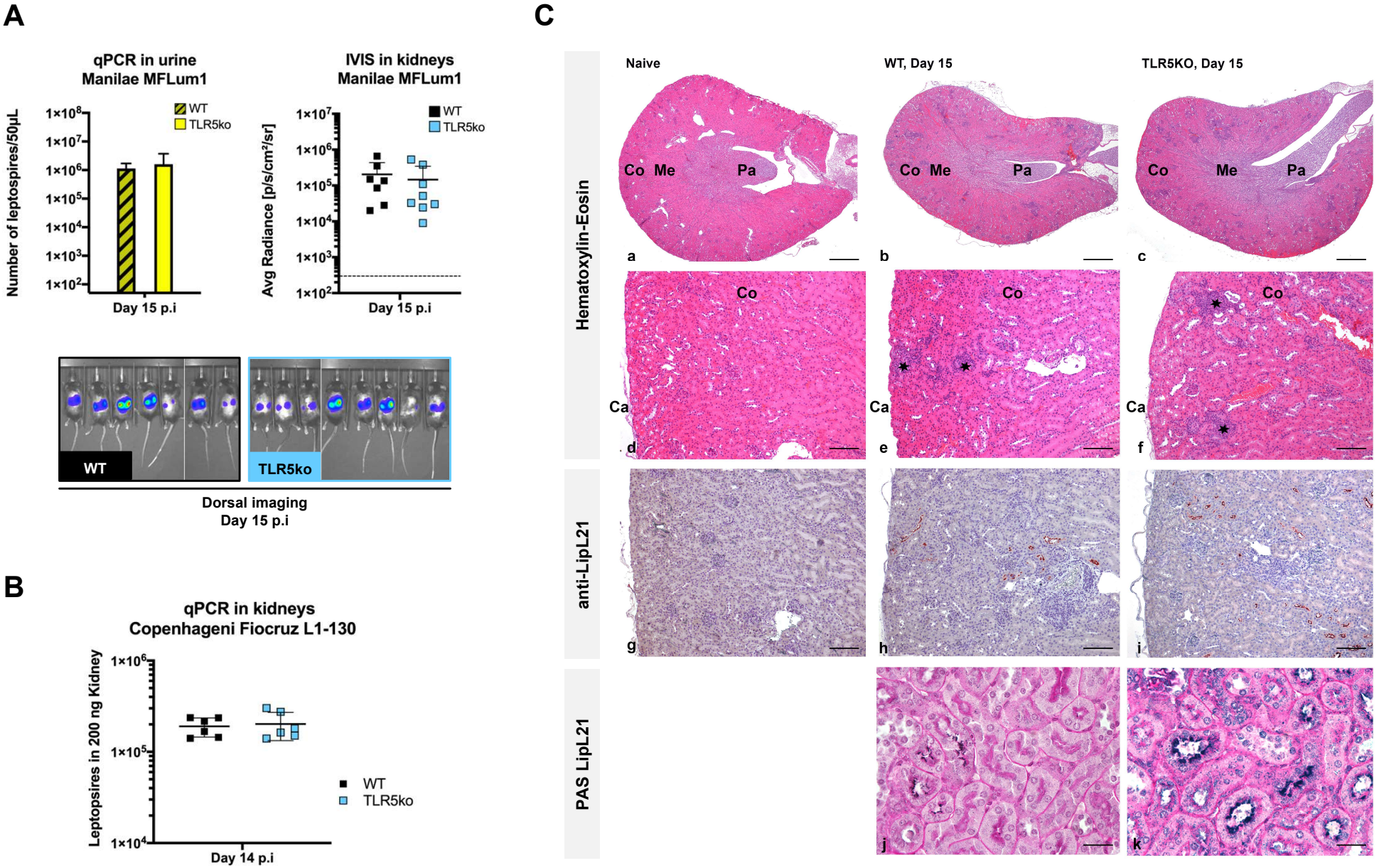


Figure 2. No difference in kidney colonization in WT and TLR5ko mice 15 days post-infection

A) Bacterial loads in urine quantified by qPCR (left panel), and quantification and images of live imaging (IVIS) (right panel) 15 days post-infection (p.i) of 7-week old female WT mice (n= 7, black) and TLR5ko mice (n= 8, blue) with 10^7 *L. interrogans* Manilae bioluminescent derivative strain MFLum1. Imaging was performed in dorsal position, 24h post shaving, on anesthetized mice and after luciferin administration. The background level of light after luciferin administration was measured on a control TLR5ko mouse injected with PBS at the time of infection (dotted line). The average radiance in individual mice gated on the whole body is shown and represented as dots; lines correspond to the mean (+/- SD) of all mice. No statistical difference was found between WT and TLR5ko mice. **B)** Bacterial loads in kidneys determined by qPCR of leptospiral DNA 14 days p.i of WT mice (n= 6, back dots) and TLR5ko (n= 6, blue dots) mice with 10^7 *L. interrogans* Copenhageni Fiocruz L1-130. Individual mice are shown and represented as dots; lines correspond to mean (+/- SD) of all mice. No statistical difference was found between WT and TLR5ko mice. **C**) Histological sections and immunolabeling of the kidneys of naive TLR5ko, infected WT and TLR5ko mice 15 days p.i with 10^7 *L. interrogans* Manilae strain MFLum1. **a-c)** Kidney, Hematoxylin-Eosin stain, Original magnification x2, Scale bar: 500 µm. Cortex (Co), Medulla (Me), Papilla (Pa), Capsule (Ca). **d-f)** Kidney cortex, Hematoxylin-Eosin stain, Original magnification x10, Scale bar: 100 µm. The asterisks indicate the focal inflammatory infiltrates. **g-i)** Anti-LipL21 labelling of leptospires in renal tubules, Original magnification x10, Scale bar: 100 µm. **j,k)** Double labelling LipL21/Periodic Acid-Schiff (PAS) to stain the PAS positive brush borders present in proximal tubules only. Original magnification x40, Scale bar: 25 µm.

Figure 3. **Heat-killed leptospires, but not live, signal through hTLR5**

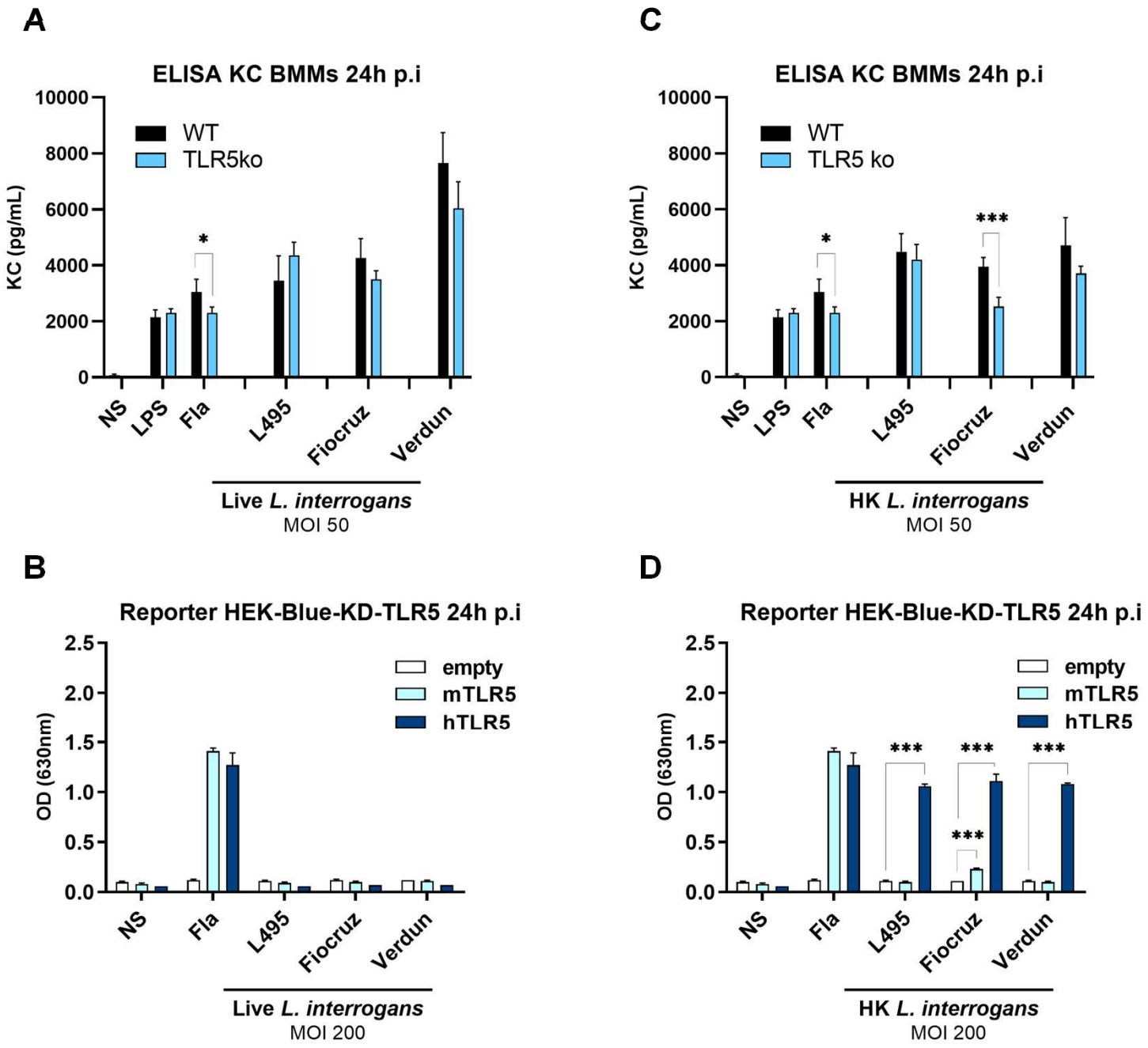


Figure 3. Heat-killed leptospires, but not live, signal through hTLR5

A,C) KC production measured by ELISA in the supernatants of BMMs from WT (black bars) and TLR5ko (blue bars) mice 24 hours post-infection with MOI 50 of either **A)** live or **C)** heat-killed (30 min, 100 ° C) different serovars of virulent *L. interrogans* (Manilae strain L495, Copenhageni strain Fiocruz L1-130, Icterohaemorrhagiae strain Verdun). LPS from *E. coli* (100 ng/mL) and unpurified Fla from *Salmonella typhimurium* (500 ng/mL) were used as controls. Data are expressed as mean (+/- SD) of technical replicates (n= 5) on pooled BMMs preparations from mice (n=3) and are representative of at least three independent experiments. Statistically significant differences between genotypes (Student t-test) are indicated. **B, D**) NF-kB reporter assay in HEK-Blue-Knock Down (KD)-TLR5 cells transfected with the mouse TLR5 (light blue bars), human TLR5 (blue bars), or empty plasmid (empty bars) and stimulated for 24 h with MOI 200 of either **B**) live or **D**) heat-killed (30 min, 100 ° C) different serovars of virulent *L. interrogans*. Unpurified Fla from *Salmonella typhimurium* (100 ng/mL) was used as control. Data are expressed as the mean (+/- SD) of technical replicates (n= 3) and are representative of at least three independent experiments. Statistically significant differences (Student t-test) are indicated.

Figure 4. A very stable protein from leptospires signal through TLR5

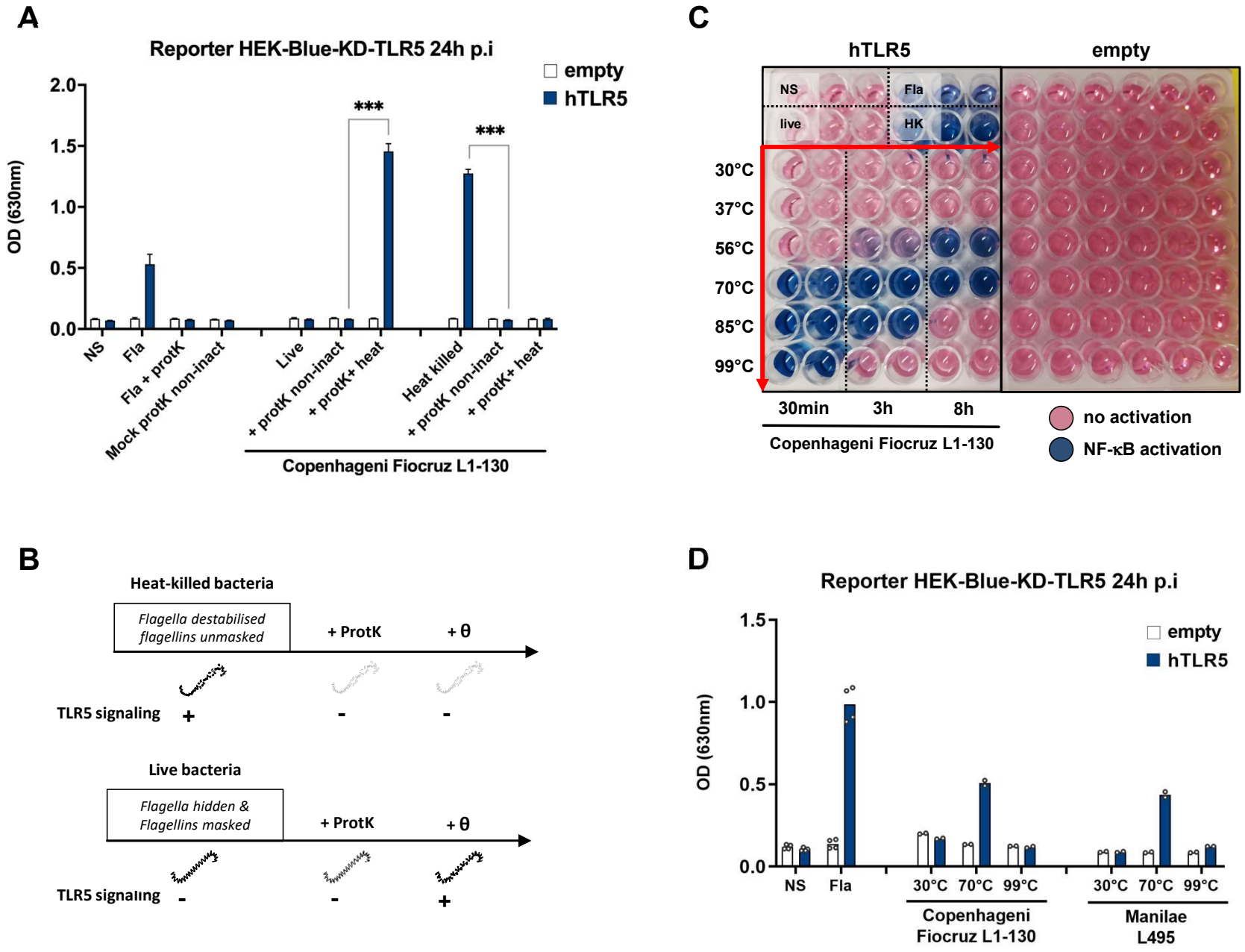


Figure 4. A very stable protein from leptospires signal through TLR5

A) NF-κB reporter assay in HEK-Blue-KD-TLR5 cells transfected with the human TLR5 (blue bars), or empty plasmid (empty bars) and stimulated with MOI 100 of either live or heat-killed (30 min, 100 ° C) *L. interrogans* Copenhageni strain Fiocruz L1-130 treated or not with Proteinase K (protK) followed or not by heat inactivation at 99° C for 30 minutes (inact or non-inact). Unpurified Fla from *Salmonella typhimurium* (100 ng/mL) was used as control. Data are expressed as the mean (+/- SD) of technical replicates (n= 3) and are representative of at least three independent experiments.
Statistically significant differences (Student t-test) are indicated. **B)** Chronogram of proteinase K experiments. **C**) Picture of NF-κB reporter assay in HEK-Blue-KD-TLR5 cells transfected with the human TLR5 or empty plasmid and stimulated with MOI 100 of live *L. interrogans* Copenhageni strain Fiocruz L1-130 incubated at various temperatures during 30 min, 3 hours, or 8 hours. Unpurified Fla from *Salmonella typhimurium* (500 ng/mL) was used as control. Picture show technical duplicate for each condition and is representative of at least three independent experiments. **D)** NF-κB reporter assay in HEK-Blue-KD-TLR5 cells transfected with the human TLR5 (blue bars) or empty plasmid (empty bars) and stimulated with MOI 100 of live *L. interrogans* Copenhageni strain Fiocruz L1-130 or Manilae L495 incubated at various temperatures during 3 hours. Unpurified Fla from *Salmonella typhimurium* (500 ng/mL) was used as control. Data are expressed as the mean of technical replicates (n= 2, shown as dots) and are representative of at least three independent experiments for Fiocruz L1-130 and two independent experiments for L495.

Figure 5. Human and bovine antimicrobial peptides unmask the leptospiral ability to signal through human and bovine TLR5 receptors

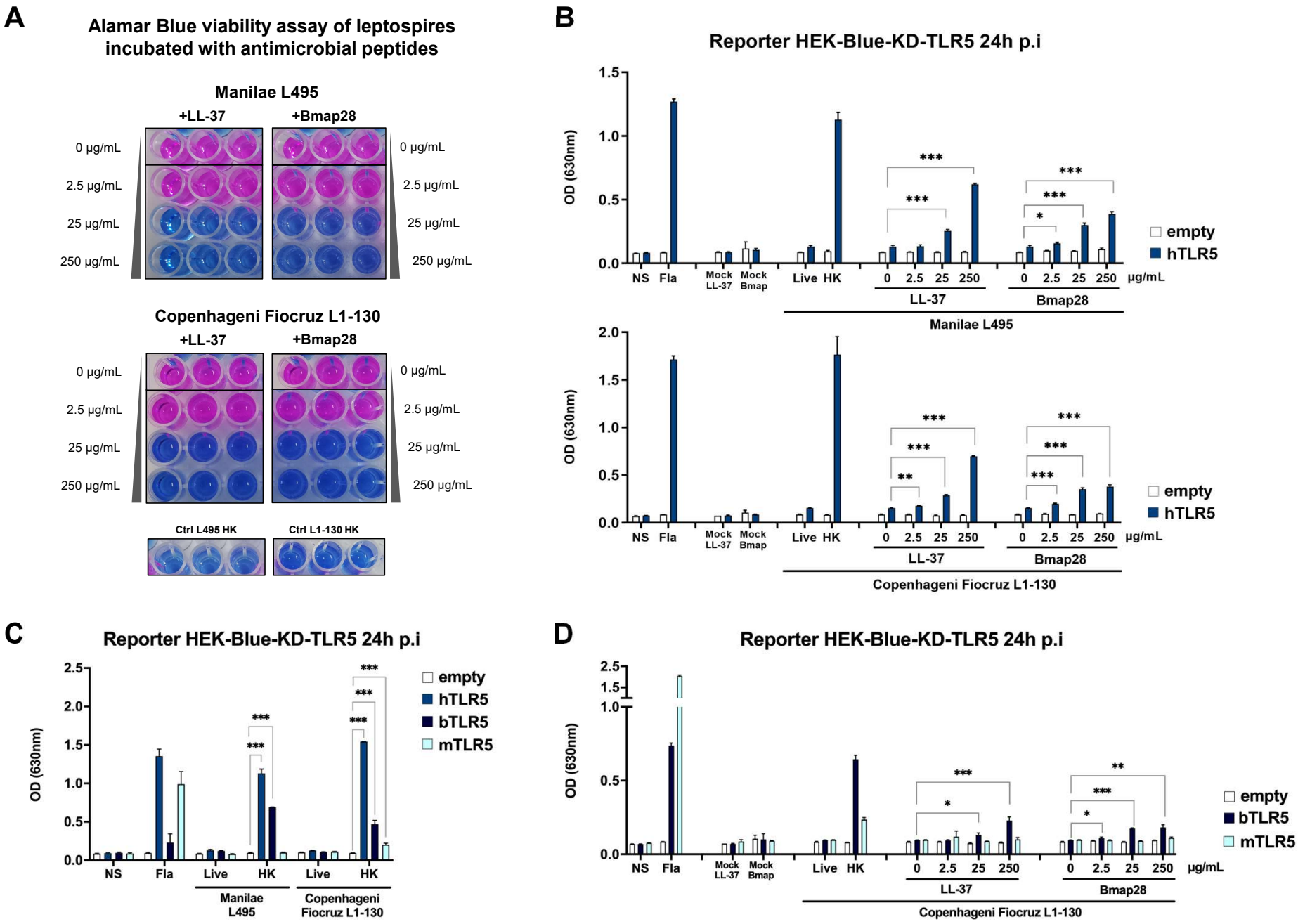


Figure 5. Human and bovine antimicrobial peptides unmask the leptospiral ability to signal through human and bovine TLR5 receptors

A) Alamar blue viability assay of leptospires (Manilae L495 or Copenhageni Fiocruz L1-130) incubated with increasing concentration (0 – 250 µg/mL) of antimicrobial peptides LL-37 or Bmap28 for two hours. Heat-killed (30 min, 100° C) leptospires are used as controls for loss of viability. Picture show technical triplicate for each condition and is representative of two independent experiments. **B)** NF-κB reporter assay in HEK-Blue-KD-TLR5 cells transfected with the human TLR5 (blue bars), or empty plasmid (empty bars) and stimulated with MOI 100 of *L. interrogans* Manilae strain L495 or Copenhageni strain Fiocruz L1-130 treated with human peptide LL-37 or bovine peptide Bmap28 at various concentration (0-250 µg/mL) for two hours before stimulation. **C)** NF-κB reporter assay in HEK-Blue-KD-TLR5 cells transfected with the human TLR5 (blue bars), bovine TLR5 (dark blue bars), mouse TLR5 (light blue bars) or empty plasmid (empty bars) and stimulated with MOI 100 of either live or heat-killed (30 min, 100 ° C) *L. interrogans* Manilae strain L495 or Copenhageni strain Fiocruz L1-130. **D)** NF-κB reporter assay in HEK-Blue-KD-TLR5 cells transfected with the bovine TLR5 (dark blue bars), mouse TLR5 (light blue bars) or empty plasmid (empty bars) and stimulated with MOI 100 of *L. interrogans* Copenhageni Fiocruz L1-130 treated with human peptide LL-37 or bovine peptide Bmap28 at various concentration (0-250 µg/mL) for two hours before stimulation. **B, C, D)** Unpurified Fla from *Salmonella typhimurium* (500 ng/mL) was used as control. Data are expressed as the mean (+/- SD) of technical replicates (n= 3), and are representative of at least three independent experiments for panels A) and C). Statistically significant differences (Student t-test) are indicated.

Figure 6. Comparison of leptospiral Flagellins and FliC structures and relation with TLR5

A

L. interrogans

	FlaBs	FlaAs
FliC	38 %	25 %

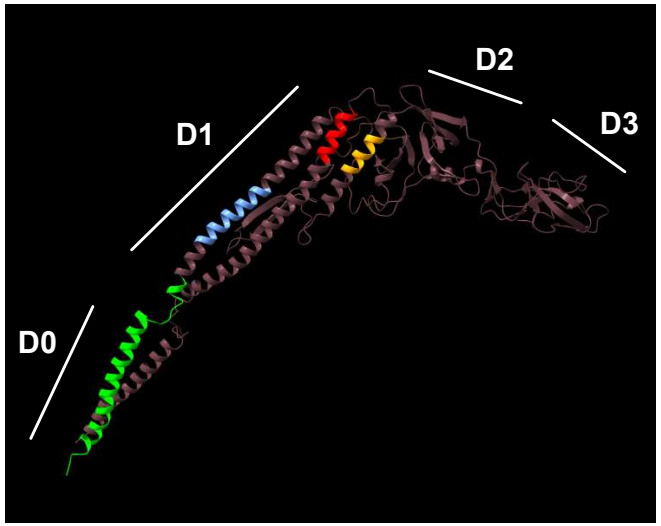
Salmonella typhimurium **FliC**

Leptospira interrogans **FlaB4**

Consensus 1
Consensus 2
Consensus 3
Stabilization region

B

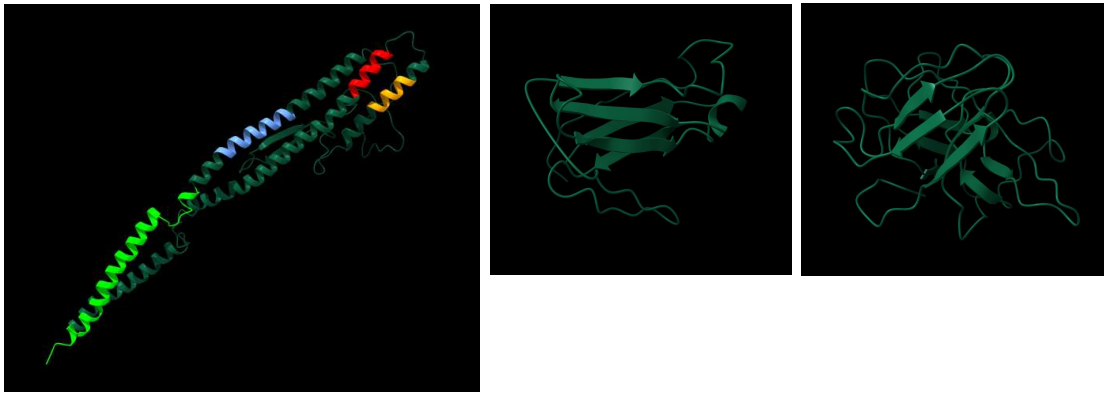
Salmonella typhimurium **FliC**



C

Leptospira interrogans strain Fiocruz

FlaB4 **FlaA1** **FlaA2**



D

			Consensus 1								Consensus 2								Consensus 3							
			*				*				*			*					*							*
	***Salmonella* FliC**		L	Q	R	V	R	E	L	T	V	Q	A	S	I	Q	N	E	I	T	Q	R	L	G	A	V	Q	N	R	F	D	S	A	I	T	N	L	G	N
FlaB1	*L. biflexa*	Patoc	I	Q	R	I	R	T	L	A	I	Q	S	M	I	Q	V	E	V	S	Q	L	L	G	A	Y	F	N	R	L	E	H	A	A	K	G	L	M	N
	L. interrogans	Fiocruz	I	Q	R	I	R	V	L	A	I	Q	S	M	I	Q	V	E	V	S	Q	L	L	G	A	Y	F	N	R	L	E	H	A	S	K	G	L	M	V
		Manilae	I	Q	R	I	R	V	L	A	I	Q	S	M	I	Q	V	E	V	S	Q	L	L	G	A	Y	F	N	R	L	E	H	A	S	K	G	L	M	V
		Verdun	I	Q	R	I	R	V	L	A	I	Q	S	M	I	Q	V	E	V	S	Q	L	L	G	A	Y	F	N	R	L	E	H	A	S	K	G	L	M	V
FlaB2	*L. biflexa*	Patoc	I	Q	R	I	R	T	L	A	I	Q	T	L	V	Q	V	E	V	S	A	L	M	G	A	Y	Q	N	R	L	E	S	T	A	K	G	L	M	G
	L. interrogans	Fiocruz	I	Q	R	I	R	V	L	A	I	Q	T	L	V	Q	V	E	V	S	A	L	M	G	A	Y	Y	N	R	L	E	Y	T	A	K	G	L	M	G
		Manilae	I	Q	R	I	R	V	L	A	I	Q	T	L	V	Q	V	E	V	S	A	L	M	G	A	Y	Y	N	R	L	E	Y	T	A	K	G	L	M	G
		Verdun	I	Q	R	I	R	V	L	A	I	Q	T	L	V	Q	V	E	V	S	A	L	M	G	A	Y	Y	N	R	L	E	Y	T	A	K	G	L	M	G
FlaB3	*L. biflexa*	Patoc	L	Q	R	L	R	E	L	S	V	Q	S	L	V	Q	L	E	V	S	A	L	L	G	A	Y	Y	N	R	L	D	L	T	L	K	S	L	S	N
	L. interrogans	Fiocruz	L	Q	R	L	R	E	L	S	I	Q	T	L	V	Q	L	E	V	D	Q	L	L	G	A	Y	Y	N	R	L	E	I	T	S	Q	G	L	Q	S
		Manilae	L	Q	R	L	R	E	L	S	I	Q	T	L	V	Q	L	E	V	D	Q	L	L	G	A	Y	Y	N	R	L	E	I	T	S	Q	G	L	Q	S
		Verdun	L	Q	R	L	R	E	L	S	I	Q	T	L	V	Q	L	E	V	D	Q	L	L	G	A	Y	Y	N	R	L	E	I	T	S	Q	G	L	Q	S
FlaB4	*L. biflexa*	Patoc	V	Q	R	V	R	V	L	A	V	Q	A	Q	I	Q	V	E	V	S	Q	L	L	G	A	Y	Y	N	R	M	E	H	A	A	K	G	L	M	N
	L. interrogans	Fiocruz	V	Q	R	I	R	V	L	A	V	Q	A	Q	I	Q	V	E	V	S	Q	L	L	G	A	Y	Y	N	R	L	E	H	A	A	K	G	L	M	N
		Manilae	V	Q	R	I	R	V	L	A	V	Q	A	Q	I	Q	V	E	V	S	Q	L	L	G	A	Y	Y	N	R	L	E	H	A	A	K	G	L	M	N
		Verdun	V	Q	R	I	R	V	L	A	V	Q	A	Q	I	Q	V	E	V	S	Q	L	L	G	A	Y	Y	N	R	L	E	H	A	A	K	G	L	M	N

Figure 6. Comparison of leptospiral Flagellins and FliC structures in relation with TLR5

A) Amino acid sequence homology average percentage between *Salmonella typhimurium* FliC (P06179) and *Leptospira interrogans* strain Fiocruz FlaBs (LIC11890, LIC11889, LIC 11532 and LIC11531) and FlaAs (LIC10788 and LIC10787) and primary structures of the flagellin proteins with TLR5 binding consensus. **B)** *In silico* (Phyre2 and Chimera softwares) prediction of *Salmonella typhimurium* FliC (P06179) structure with the four described domains and with positions of the TLR5 binding consensus: 1 (red), 2 (yellow) and 3 (light blue) and stabilization region (light green) highlighted. **C)** *In silico* (Phyre2 and Chimera softwares) prediction of *Leptospira interrogans* strain Fiocruz FlaB4 (LIC11531) with the positions of the TLR5 binding consensus and stabilization region highlighted, FlaA1 (LIC10788), FlaA2 (LIC10787). **D)** Clustal (MEGA software) alignment of the amino acid sequences for the TLR5 binding consensus regions of: *Salmonella enterica* FliC (GeneBank QDQ31983.1), *L. biflexa* (strain Patoc) FlaB1 (LEPBIa2133), FlaB2 (LEPBIa2132), FlaB3 (LEPBIa1872) and FlaB4 (LEPBIa1589), *L. interrogans* (strain Fiocruz L1-130) FlaB1 (LIC18890), FlaB2 (LIC11889), FlaB3 (LIC11532) and FlaB4 (LIC11531), *L. interrogans* (strain L495) FlaB1 (LMANv2_260016), FlaB2 (LMANv2_260015), FlaB3 (LMANv2_590024) and FlaB4 (LMANv2_590023), *L. interrogans* (strain Verdun) FlaB1 (AKWP_v1_110429), FlaB2 (AKWP_v1_110428) and FlaB3 (AKWP_v1_110068) and FlaB4 (AKWP_v1_110067).

Figure 7. **FlaB subunits, not FlaAs nor Fcps, contribute to the signaling**

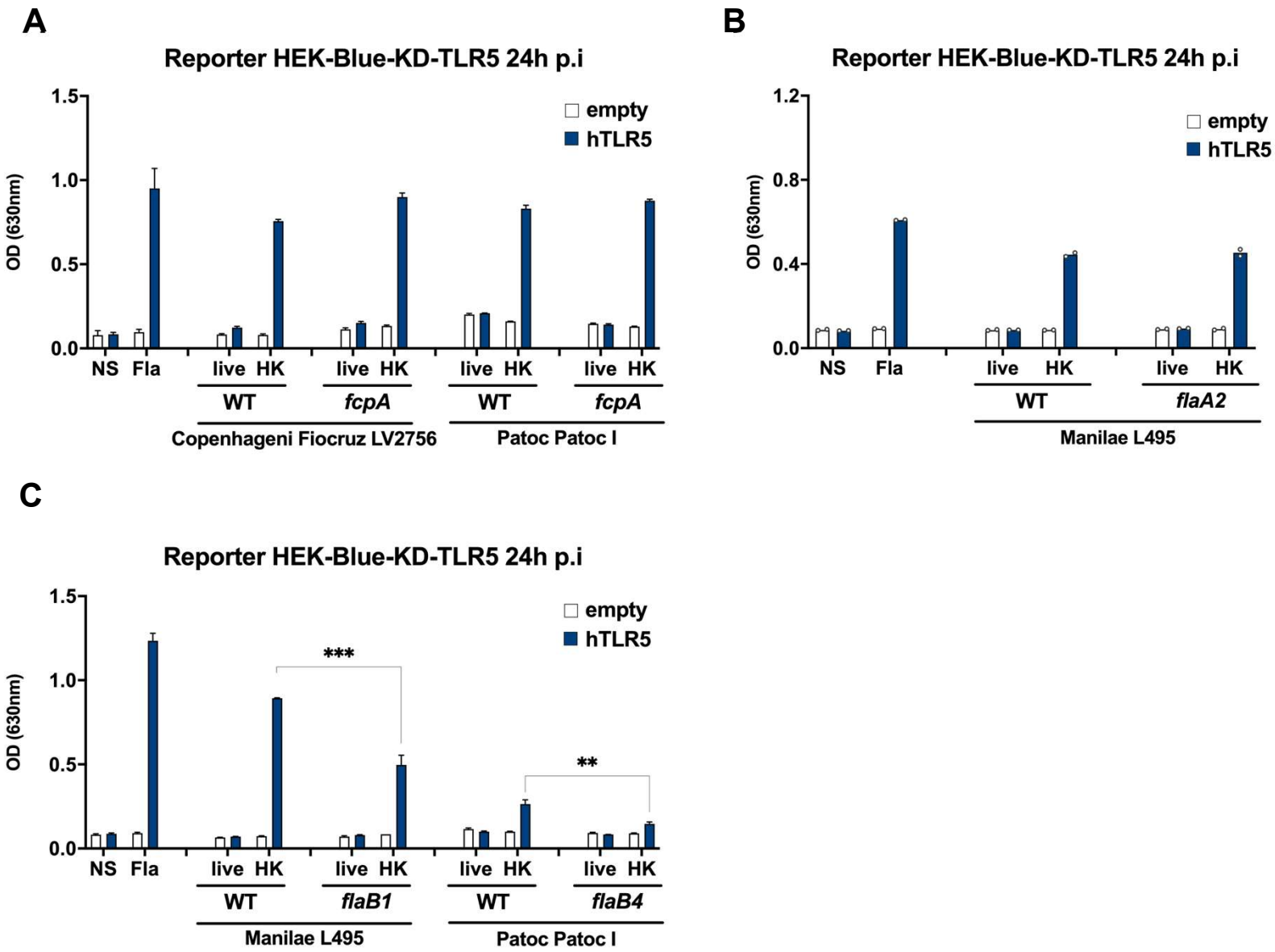


Figure 7. FlaB subunits, not FlaAs nor Fcps, contribute to the signaling

A) NF-κB reporter assay in HEK-Blue-KD-TLR5 cells transfected with the human TLR5 (blue bars), or empty plasmid (empty bars) and stimulated with MOI 100 of either live or heat-killed (30 min, 100 ° C) *L. interrogans* Copenhageni Fiocruz LV2756 WT or ΔFcpA, Patoc Patoc I WT or ΔFcpA. Unpurified Fla from *Salmonella typhimurium* (500 ng/mL) was used as control. Data are expressed as the mean (+/- SD) of technical replicates (n= 3) and are representative of at least three independent experiments. **B)** NF-κB reporter assay in HEK-Blue-KD-TLR5 cells transfected with the human TLR5 (blue bars), or empty plasmid (empty bars) and stimulated with MOI 100 of either live or heat-killed (30 min, 100 ° C) *L. interrogans* Manilae L495 WT or ΔFlaA2. Unpurified Fla from *Salmonella typhimurium* (500 ng/mL) was used as control. Data are expressed as the mean of technical duplicates (n= 2, shown as dots). **C)** NF-κB reporter assay in HEK-Blue-KD-TLR5 cells transfected with the human TLR5 (blue bars), or empty plasmid (empty bars) and stimulated with MOI 100 of either live or heat-killed (30 min, 100 ° C) *L. interrogans* Manilae L495 WT or ΔFlaB1 and Patoc Patoc I WT or ΔFlaB4. Data are expressed as the mean (+/- SD) of technical replicates (n= 3) and are representative of at least three independent experiments. Statistically significant differences (Student t-test) are indicated.

Figure 8. **FlaBs mRNA are upregulated in stationary phase, which may impact TLR5 recognition, and dowregulated *in vivo***

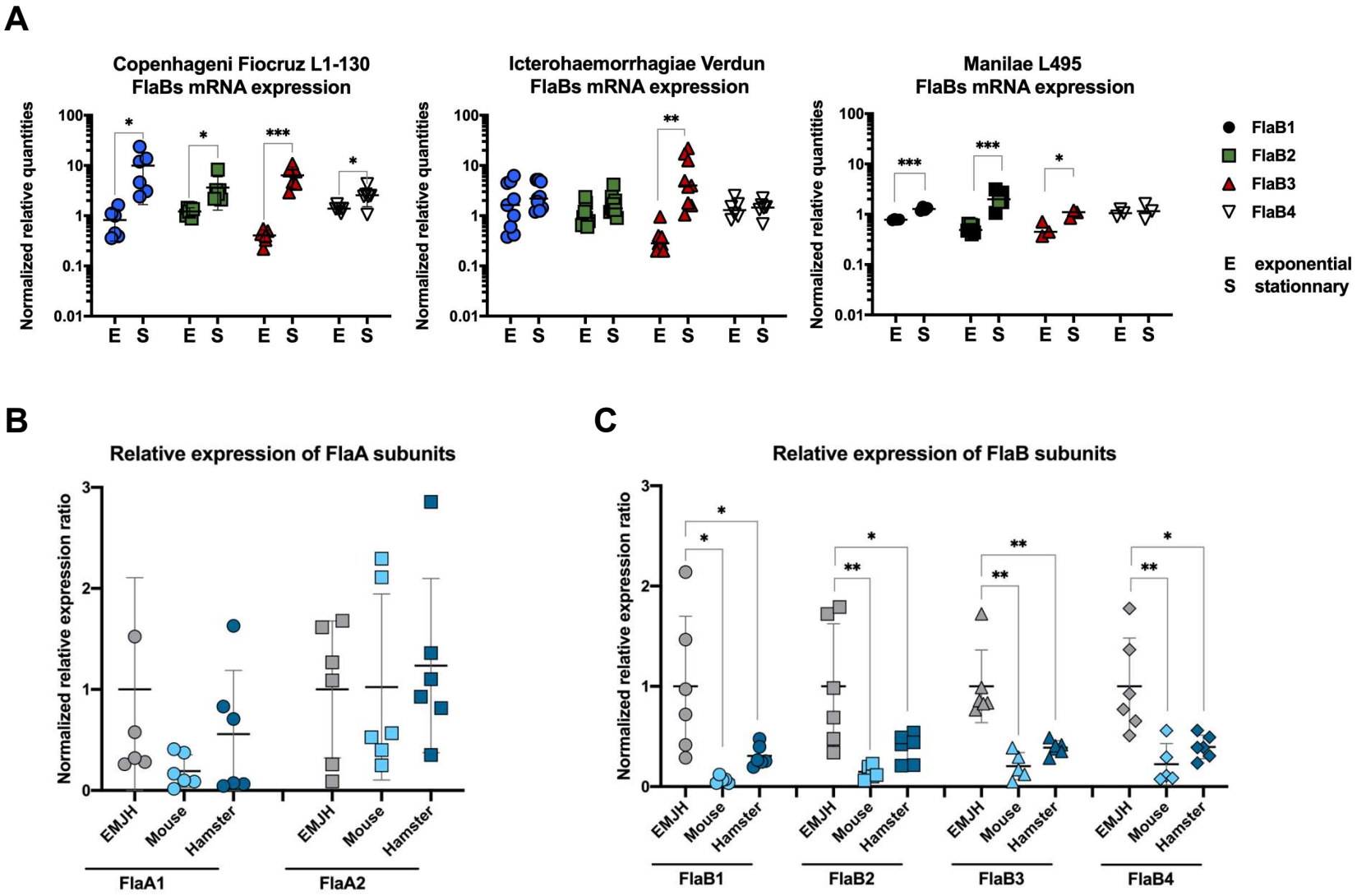
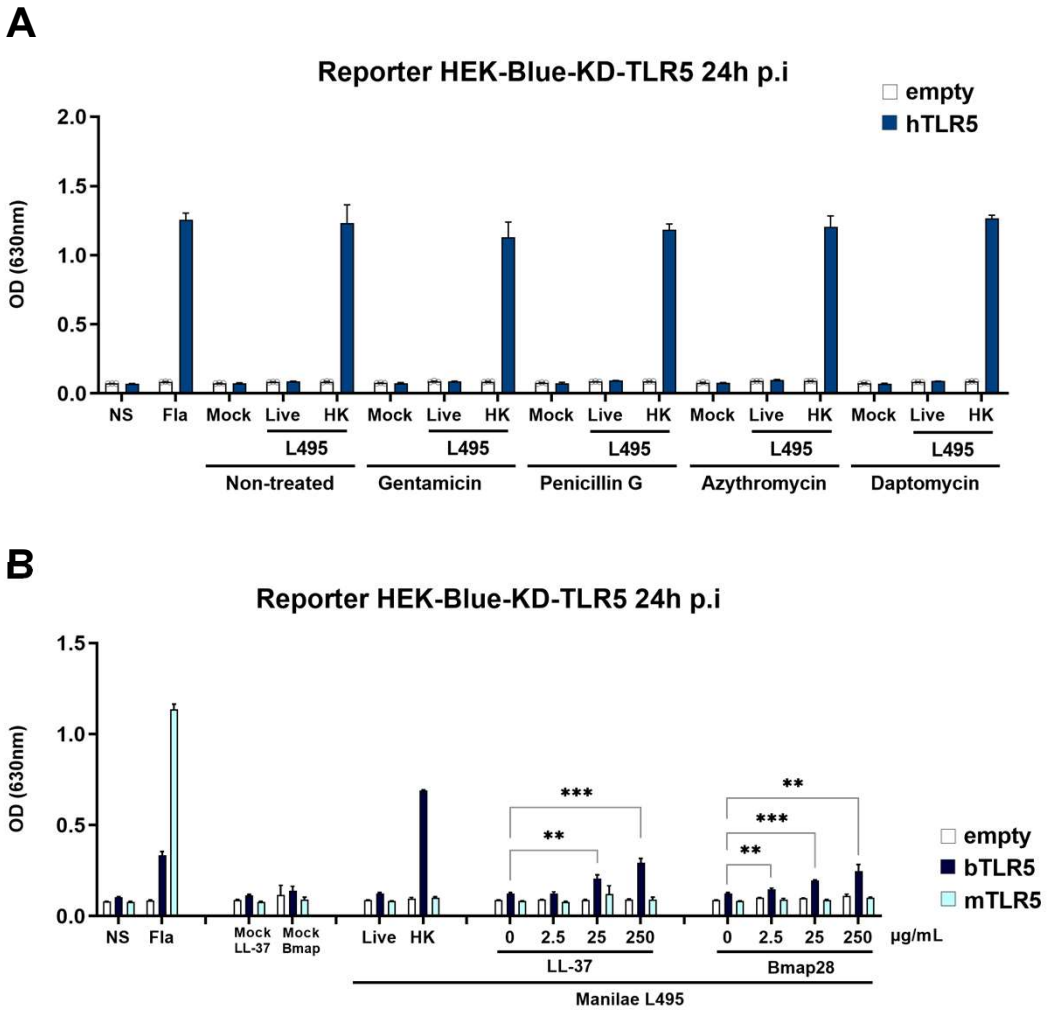


Figure 8. FlaBs mRNA are upregulated in stationary phase and downregulated *in vivo*

A) *In vitro* FlaBs mRNA expression in *L. interrogans* Copenhageni Fiocruz L1-130, Icterohaemorrhagiae Verdun and Manilae L495 at the exponential (E) and stationary (S) phase. Data of RT-qPCR are expressed as the relative mRNA quantities normalized to the expression of the *lipl41* mRNA. Technical replicates are represented as dots and lines correspond to mean (+/- SD) of replicates ($3 < n < 9$). Statistically significant differences (Student t-test) are indicated. **B)** *In vivo* FlaAs and **C)** FlaBs mRNA expression in blood of infected mice (n=5, light blue) and hamsters (n=5, dark blue), 24 h post intraperitoneal infection with $2x10^8$ virulent *L. interrogans* Icterohaemorrhagiae strain Verdun, compared with mRNA expression in culture in EMJH at 30° C. Data of RT-qPCR are expressed as the ratio of mRNA quantities relatives to the EMJH control. Individual animals are represented as dots and lines correspond to mean (+/- SD) of all animals. Statistically significant differences (Student t-test) are indicated.

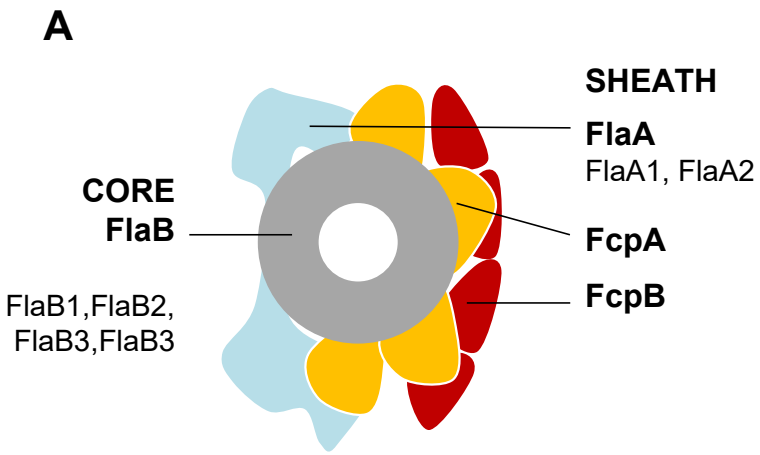
Sup Figure 1. **Not antibiotics but antimicrobial peptides reveal the L495 ability to activate TLR5**



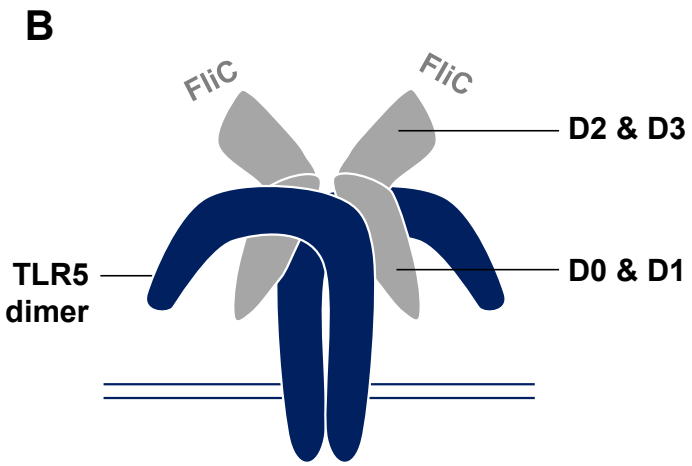
Sup Figure 1. Not antibiotics but antimicrobial peptides reveal the L495 ability to activate TLR5

A) NF-κB reporter assay in HEK-Blue-KD-TLR5 cells transfected with the human TLR5 (blue bars), or empty plasmid (empty bars) and stimulated with MOI 100 of *L. interrogans* Manilae strain L495 treated with gentamicin (50 µg/mL), penicillin G (10 µg/mL), azithromycin (10 µg/mL) or daptomycin (1 µg/mL) for 4 h before stimulation. Data are expressed as the mean (+/- SD) of technical replicates (n= 3). **B)** NF-κB reporter assay in HEK-Blue-KD-TLR5 cells transfected with the bovine TLR5 (dark blue bars), mouse TLR5 (light blue bars) or empty plasmid (empty bars) and stimulated with MOI 100 of *L. interrogans* Manilae strain L495 treated with human peptide LL-37 or bovine peptide Bmap28 at various concentration (0-250 µg/mL) for two hours before stimulation. Unpurified Fla from *Salmonella typhimurium* (500 ng/mL) was used as control. Data are expressed as the mean (+/- SD) of technical replicates (n= 3) and are representative of at least three independent experiments. Statistically significant differences (Student t-test) are indicated.

Sup Figure 2. **Schematics of leptospiral filament and FliC association with TLR5**



Adapted from « An asymmetric sheath controls flagellar supercoiling and motility in the leptospira spirochete. Gibson *et al*., *eLife* (2020) »



Adapted from « Structural basis of TLR5-Flagellin Recognition and Signaling. Yoon *et al*., *Science* (2012) »

Sup Figure 2. Schematics of leptospiral filament and FliC association with TLR5

Schematic representations of **A)** the leptospiral flagellin structure adapted from Gibson *et al.* 2020 and **B)** the interaction of FliC subunits with TLR5 inducing dimerization of the receptors adapted from Yoon *et al.* 2012.

Sup Figure 3. BLAST analyses between leptospiral flagellin subunits and species

A

BLAST-P identity % **Fiocruz**	FlaB1	FlaB2	FlaB3
FlaB4	72%	67%	51%
FlaB1	/	72%	55%
FlaB2	/	/	57%

BLAST-P identity % **Patoc**	FlaB1	FlaB2	FlaB3
FlaB4	69%	64%	49%
FlaB1	/	68%	52%
FlaB2	/	/	55%

B

BLAST-P (Identity %) **FlaB1**	Manilae	Verdun	Patoc
Fiocruz	100%	100%	62%

BLAST-P (Identity %) **FlaB4**	Manilae	Verdun	Patoc
Fiocruz	99%	100%	92%
Manilae	/	99%	91%

BLAST-P (Identity %) **FlaB3**	Manilae	Verdun	Patoc
Fiocruz	99%	100%	87%
Manilae	/	99%	87%

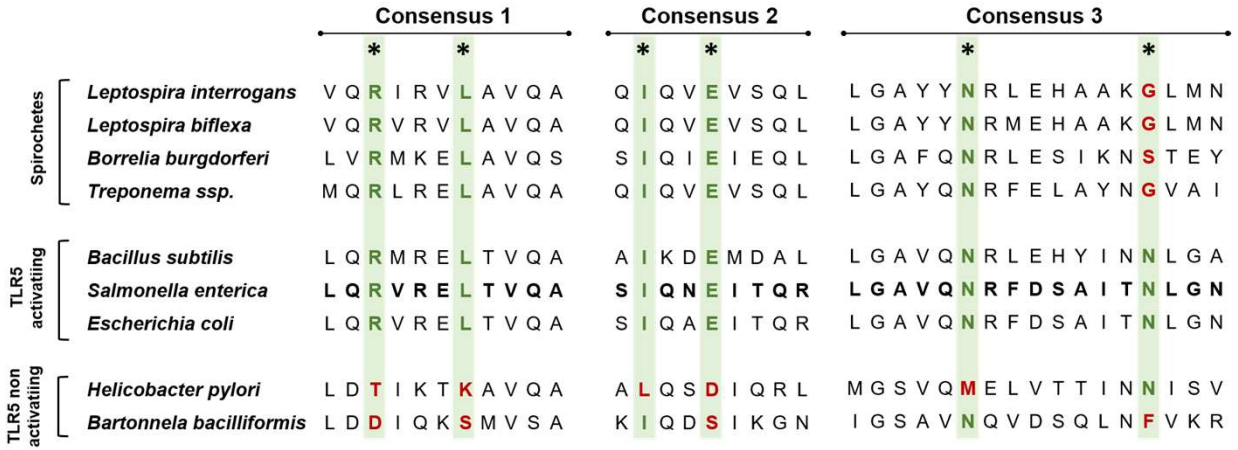
BLAST-P (Identity %) **FlaB2**	Manilae	Verdun	Patoc
Fiocruz	100%	100%	78%

Sup Figure 3. BLAST analyses between flagellin subunits and species

A-B) Amino acid sequence homology percentage between **A)** *Leptospira interrogans* strain Fiocruz FlaBs (LIC18890, LIC11889, LIC11532, LIC11531) or *Leptospira biflexa* strain Patoc FlaBs (LEPBIa2133, LEPBIa2132, LEPBIa1872, LEPBIa1589), **B)** *Leptospira interrogans* FlaB1 of all serotypes (Fiocruz LIC11890, Manilae LMANv2_260016, Verdun AKWP_v1_110429, Patoc LEPBIa2133), FlaB2 of all serotypes (Fiocruz LIC11889 , Manilae LMANv2_260015, Verdun AKWP_v1_110428, Patoc LEPBIa2132), FlaB3 of all serotypes (Fiocruz LIC11532, Manilae LMANv2_590024, Verdun AKWP_v1_110068, Patoc LEPBIa1872) and FlaB4 of all serotypes (Fiocruz LIC11531, Manilae LMANv2_590023, Verdun AKWP_v1_110067, Patoc LEPBIa1589).

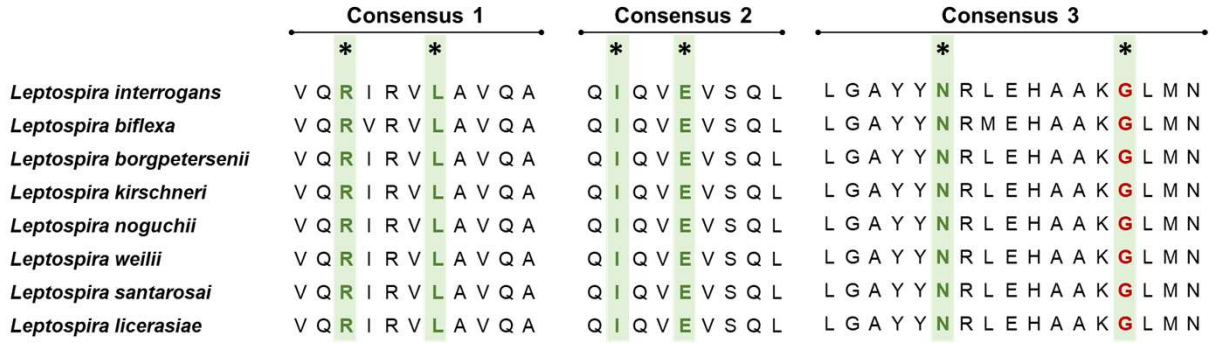
Sup Figure 4. TLR5 binding and consensus sites in different species

A



Adapted from « A conserved TLR5 binding and activation hot spot on flagellin. Song *et al*., *Sci. Reports* (2017) »

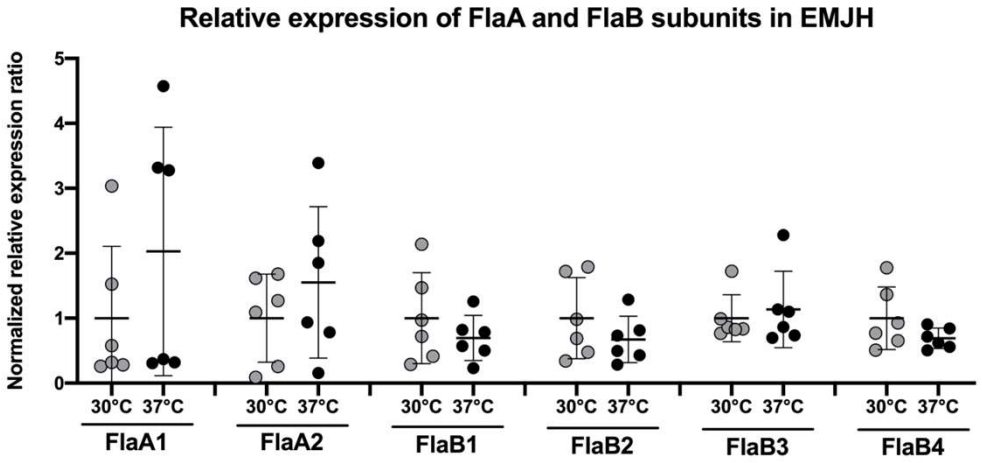
B



Sup Figure 4. TLR5 binding and consensus sites in different species

A-B) Clustal (MEGA software) alignment of the amino acid sequences for the TLR5 binding consensus regions of: **A)** *Leptospira interrogans* strain Fiocruz FlaB4 (LIC11531), *Leptospira biflexa* strain Patoc FlaB4 (LEPBIa1589), *Borrelia burgdorferi* (GeneBank CAA45011.1), *Treponema* ssp. (GeneBank AIW88993.1), *Bacillus subtilis* strain W23 (GeneBank ADM39502.1), *Salmonella enterica* subsp. *enterica* serovar Typhimurium (GeneBank QDQ31983.1), *Escherichia coli* strain 0157:H7 (KKF82802.1), *Helicobacter pylori* strain J99 (GeneBank AKE81874.1) and *Bartonnela bacilliformis* (GeneBank AAA22899.1) and **B)** FlaB4s or homologs of *L. interrogans* Copenhageni strain Fiocruz L1-130 (LIC11531), *L. biflexa* Patoc strain Patoc (LEPBIa1589), *L. borgpetersenii* Hardjo-bovis strain JB197 (Q04TC0_LEPBJ), *L. kirschneri* strain H1 (A0A0E2B0X3_9LEPT), *L. noguchii* Autumnalis strain ZUN142 (M6UC47_9LEPT), *L. weilii* Topaz strain LT2116 (M3G645_9LEPT), *L. santarosai* Arenal strain MAVJ 401 (M6JKS8_9LEPT) and *L. licerasiae* Varillal strain VAR 010 (I0XRK5_9LEPT).

Sup Figure 5. **Temperature of culture medium does not impact FlaAs and FlaBs regulation**

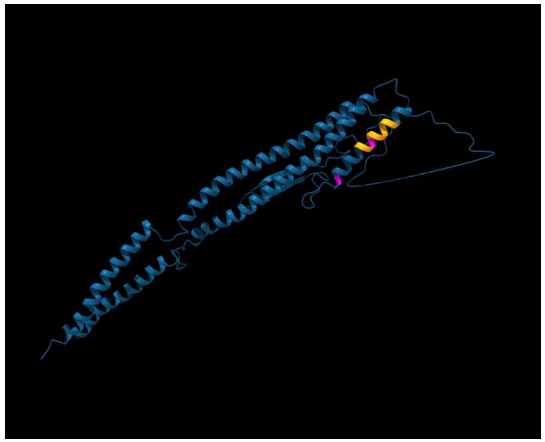


Sup Figure 5. Temperature of culture medium does not alter FlaAs and FlaBs regulation

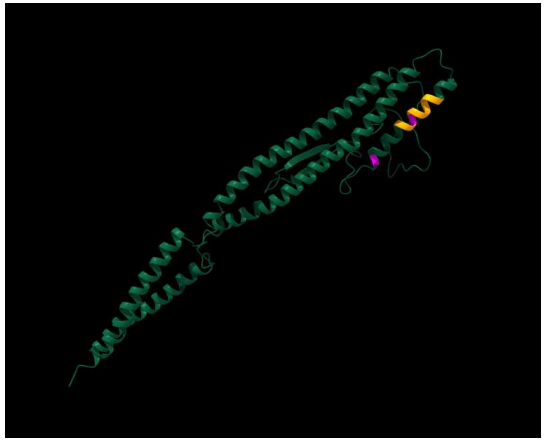
FlaAs and FlaBs mRNA expression in culture in EMJH at 30° C or 37° C. Data of RT-qPCR are expressed as the ratio of mRNA quantities relatives to the EMJH 30° C control. Technical replicates are represented as dots and lines correspond to mean (+/- SD) of all replicates.

Sup Figure 6. Glycosylation sites on FlaBs

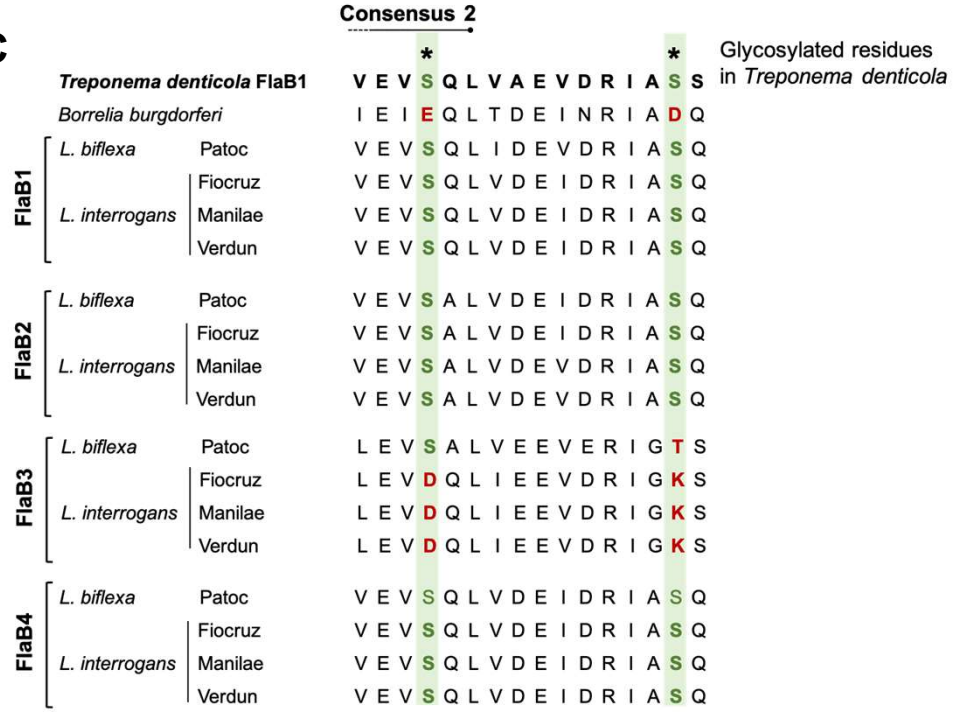
A *Treponema pallidum* **FlaB1**



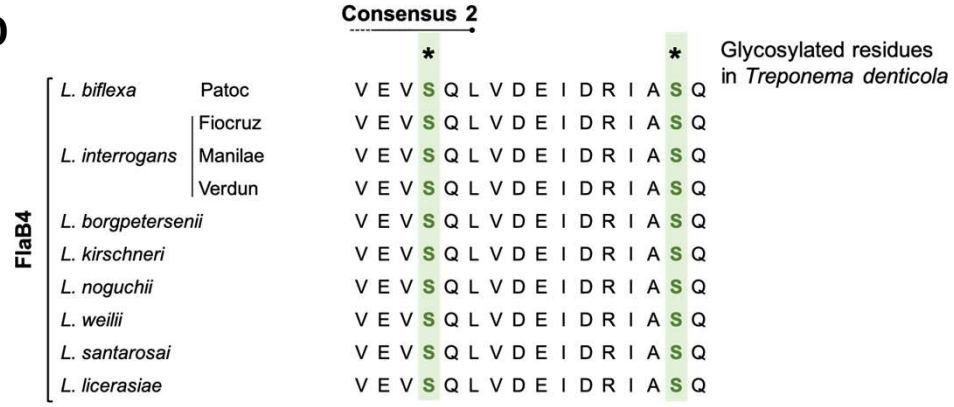
B *Leptospira interrogans* **FlaB4**



C



D



Sup Figure 6. Glycosylation sites on FlaBs

A-B) *In silico* (Phyre2 and Chimera softwares) prediction of **A)** *Treponema pallidum* strain Nichols FlaB1 (P21990) and **B)** *L.interrogans* Copenhageni strain Fiocruz L1-130 FlaB4 (LIC11531) with TLR5 binding consensus 2 (yellow) and potential glycosylation positions (pink) highlighted. **C-D)** Clustal (MEGA software) alignment of the amino acid sequences for region with potential glycosylations of: **C)** *Treponema denticola* FlaB1 (GeneBank WP_010697276.1), *Borrelia burgdorferi* (GeneBank CAA45011.1), *L. biflexa* Patoc strain Patoc FlaB1 (LEPBIa2133), FlaB2 (LEPBIa2132), FlaB3 (LEPBIa1872), FlaB4 (LEPBIa1589), *L. interrogans* Copenhageni strain Fiocruz L1-130 FlaB1 (LIC18890), FlaB2 (LIC11889), FlaB3 (LIC11532), FlaB4 (LIC11531), Manilae strain L495 FlaB1 (LMANv2_260016), FlaB2 (LMANv2_260015), FlaB3 (LMANv2_590024), FlaB4 (LMANv2_590023), and Icterohaemorrhagiae strain Verdun FlaB1 (AKWP_v1_110429), FlaB2 (AKWP_v1_110428) and FlaB3 (AKWP_v1_110068), FlaB4 (AKWP_v1_110067), and **D)** FlaB4s or homologs of *L. biflexa* Patoc strain Patoc (LEPBIa1589), *L. interrogans* Copenhageni strain Fiocruz L1-130 (LIC11531), Manilae strain L495 (LMANv2_590023), Icterohaemorrhagiae strain Verdun (AKWP_v1_110067), *L. borgpetersenii* Hardjo-bovis strain JB197 (Q04TC0_LEPBJ), *L. kirschneri* strain H1 (A0A0E2B0X3_9LEPT), *L. noguchii* serovar Autumnalis strain ZUN142 (M6UC47_9LEPT), *L. weilii* serovar Topaz strain LT2116 (M3G645_9LEPT), *L. santarosai* serovar Arenal strain MAVJ 401 (M6JKS8_9LEPT) and *L. licerasiae* serovar Varillal strain VAR 010 (I0XRK5_9LEPT).