

Supplementary Information

***Leptospira* surface adhesin (Lsa21) induces Toll like receptor 2 and 4 mediated inflammatory responses in macrophages**

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The supplementary file contains Figure S1, Figure S2 and its legend and Table 1.

Figure S1

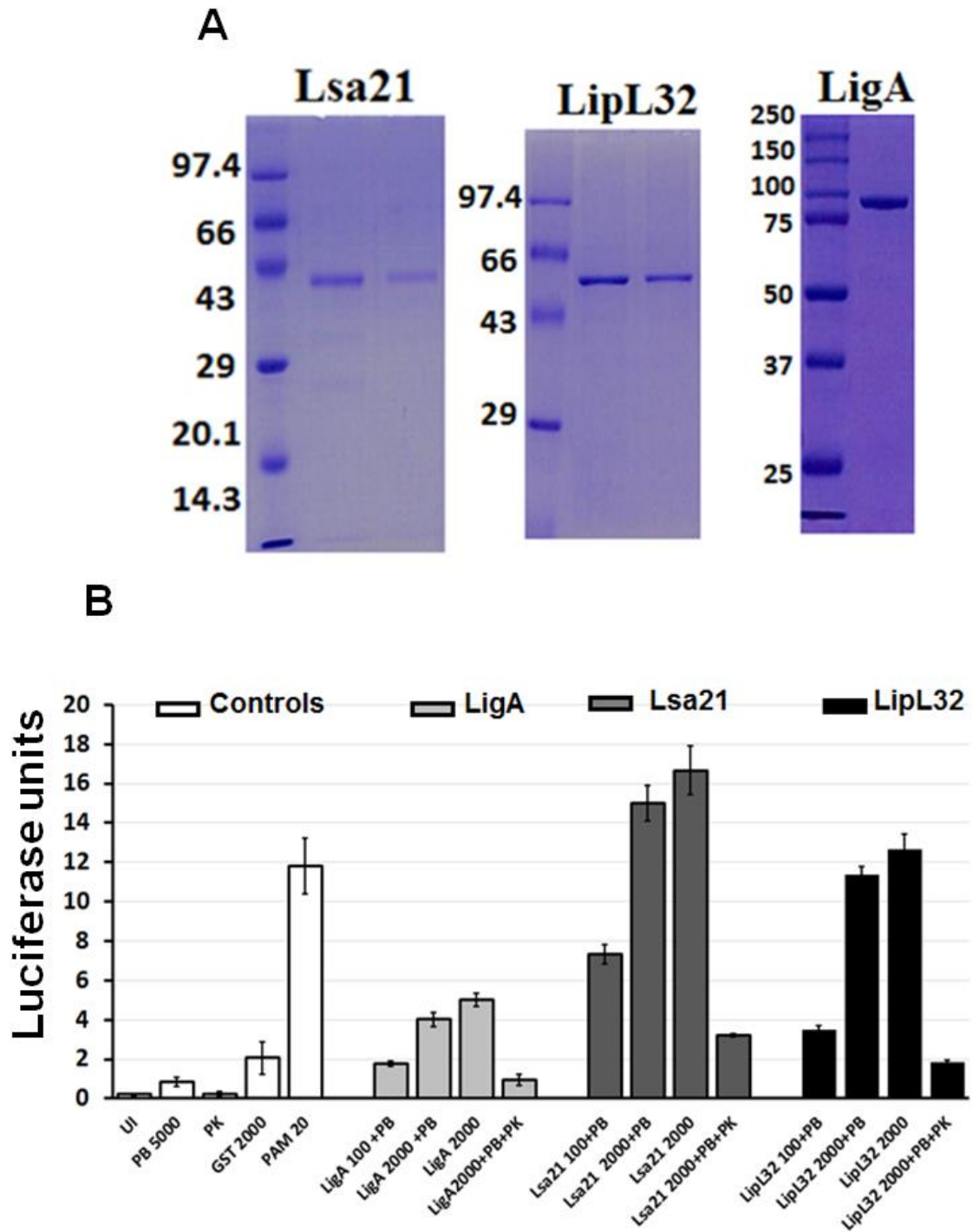


Figure S1. Purification and testing of TLR activity of recombinant *Leptospira* surface proteins.

(A) GST fusion *Leptospira* surface proteins (Lsa21, LipL32 and LigAvar) were purified using standard procedure. (B) Screening of *Leptospira* proteins for TLR2 activity on HEK293 cell lines. TLR2 activity measured by using Luciferase assay after stimulation with the proteins as described in material and methods.

Figure S2

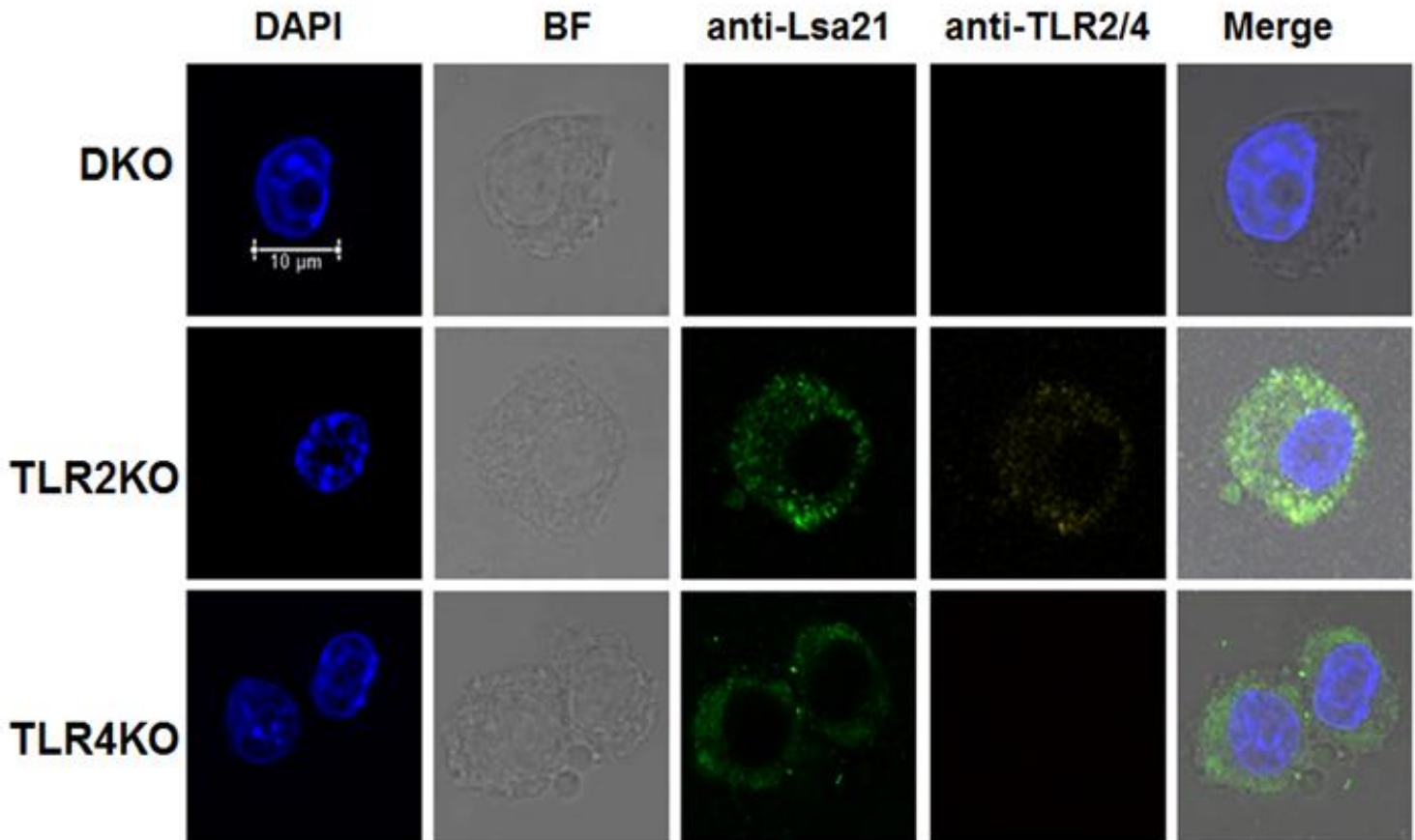


Figure S2. *rLsa21* interacts with *TLR2* and *TLR4*. $TLR2^{-/-}$, $TLR4^{-/-}$ and $TLR2^{-/-}/4^{-/-}$ mouse macrophage cell lines were cultured in cell imaging dishes and then treated with *rLsa21* (2 μg/ml) for 30 min. After washing, the cells were fixed with 4% PFA and then sequentially incubated with mouse anti-*rLsa21* serum and FITC Goat anti-mouse IgG, Alexa Fluor® 647-rabbit anti-mouse *TLR2* and PE-rabbit anti-mouse *TLR4*. After washing, mounting medium (VECTASHIELD® with DAPI) was added and samples were viewed and photographed with confocal fluorescence microscopy. Data are representative of three independent experiments.

Table1. Primers used for RT-PCR

S.NO	Gene	Primer Sequence
1	Beta actin	F- CACCCACACTGTGCCCATCTACGA R- GGATGCCACAGGATTCCATACCCA
2	IL-1b	F- GCCTTGGGCCTCAAAGGAAAGAATC R- GGAAGACACAGATTCCATGGTGAAG
3	Il6	F- TGGAGTCACAGAAGGAGTGGCTAAG R- TCTGACCACAGTGAGGAATGTCCAC
4	TNF- α	F- ATAGCTCCCAGAAAAGCAAGC R- CACCCCGAAGTTCAGTAGACA
5	CCR5	F - AACTCAGTATCATTCTGG R- GGATCAGGCTCAAGATGACC
6	Mcp1	F- GGAAAATGGATCCACACCTTGC R- TCTCTTCCCTCCACCACCATGCAG
7	Tlr2	F- CTCCTGAAGCTGTTGCGTTAC R- GCTCCCTTACAGGCTGAGTTC
8	Tlr4	F- TCGCCTTCTTAGCAGAAACAC R- GCCTTAGCCTCTTCTCCTTC
9	Cox2	F- TCTGGAACATTGTGAACAACATC R- AAGCTCCTTATTTCCCTTCACAC
10	Cxcr4	F- GAAGTGGGGTCTGGAGACTATG R- AGGGGAGTGTGATGACAAAGAG
11	Icam2	F- TGCTGTTCTTATTTGTGACATCTG R- TGTATTGAGGCTAAAAAGGAGAGG
12	CCL2	F- ACGTGTTGGCTCAGCCAGA R- ACTACAGCTTCCTTTGGGACACC
13	iNOS	F- CAGCCCAACAATACAAGATGACCC R- CAGTTCCGAGCGTCAAAGACCTGC
14	IFN-g	F- ACTCAAGTGGCATAGATGTGGAAG R- GACGCTTATGTTGTTGCTGATGG
15	CCR4	F- ATCCTGAAGGACTTCAAGCTCCA R- AGGTCTGTGCAAGATCGTTTCATGG
16	CXCR3	F- GCCGGAGCACCAGCCAAGCCAT R- AGGTGGAGCAGGAAGGTGTC
17	CXCL10	F- CATGGTCCTGAGACAAAAGT R- TGATGACACAAGTTCTTCCA
18	STAT 1	F- AGGGGCCATCACATTCACAT R- AGATACTTCAGGGGATTCTC
19	CCL3	F- ACTGCCTGCTGCTTCTCCTACA R- AGGAAAATGACACCTGGCTGG