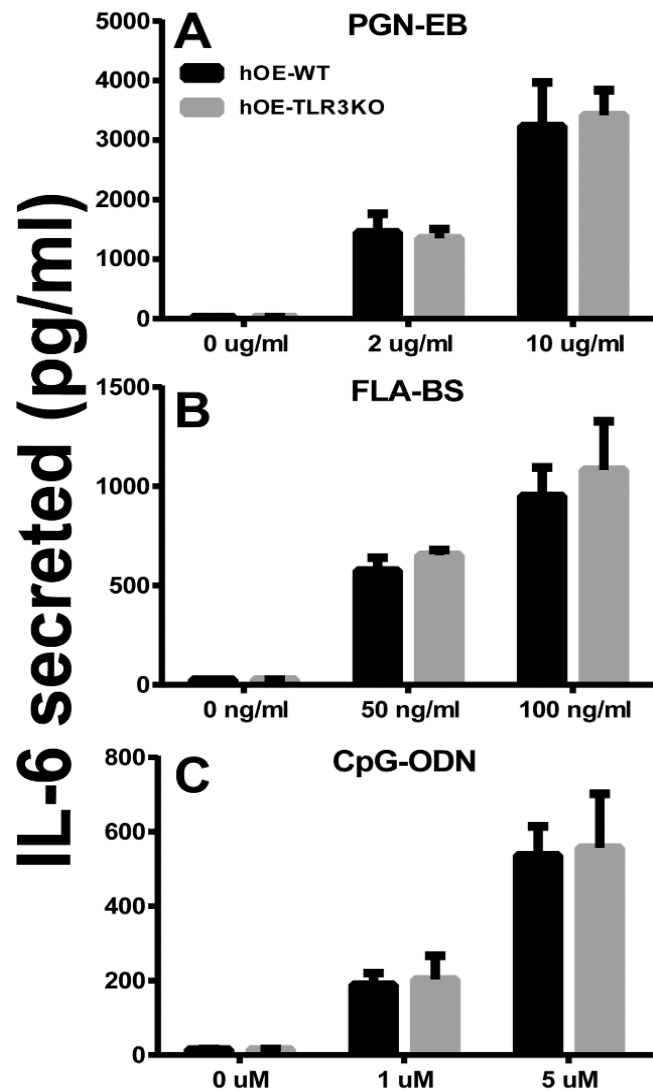


B

Target ID	gRNA sequence + PAM	Species	Gene	Gene ID	RefSeq	Exon
HS0000274415	CCACCTGAAGTT GACTCAGGTA	Human	TLR3	7098	NM_003265	2
HS0000274416	CCAACCTCACAA GGTATAGCCA	Human	TLR3	7098	NM_003265	2
HS0000274440	CAGGGTGTTTTTC ACGCAATTGG	Human	TLR3	7098	NM_003265	4

1
2
3
4
5
6

FIG S1. (A) Vector map of the all-in-one lentivirus CRISPR vector. (B) Selected TLR3-specific gRNA sequences incorporated into lentiviral particles for disruption of TLR3 gene expression.



7

8

9

FIG S2. ELISA from supernatants harvested at 24h post-infection from WT

10

vs. TLR3KD hOE cells treated with various TLR PAMPs. ELISA was used to measure

11

the synthesis of IL-6 in WT and TLR3KD hOE cells 24hrs after the addition of (A) 0, 2 and

12

10µg/ml peptidoglycan purified from *E.coli* O111:B4 (**PGN-EB**); (B) 0, 50, and 100ng/ml

13

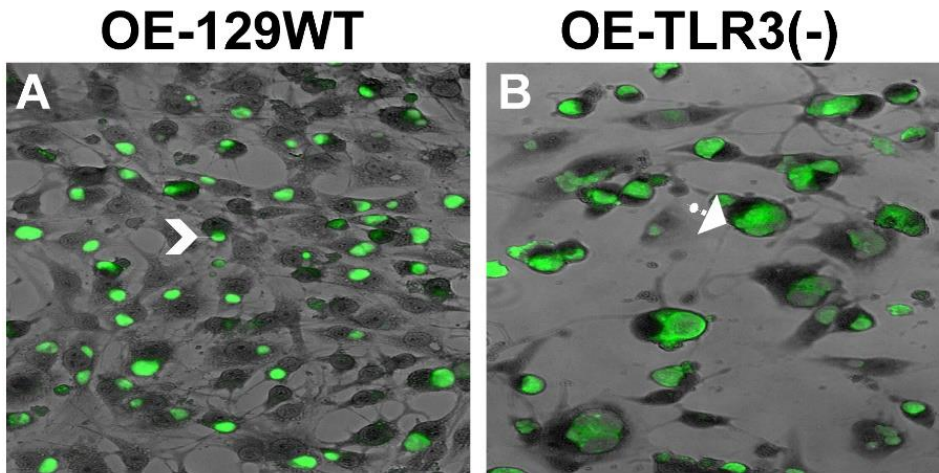
ultrapure flagellin isolated from the Gram-positive *B. subtilis* (**FLA-BS**); and 0, 1, and 5µM

14

of synthetic oligonucleotides containing unmethylated CpG dinucleotides (**CpG-ODN**).

15

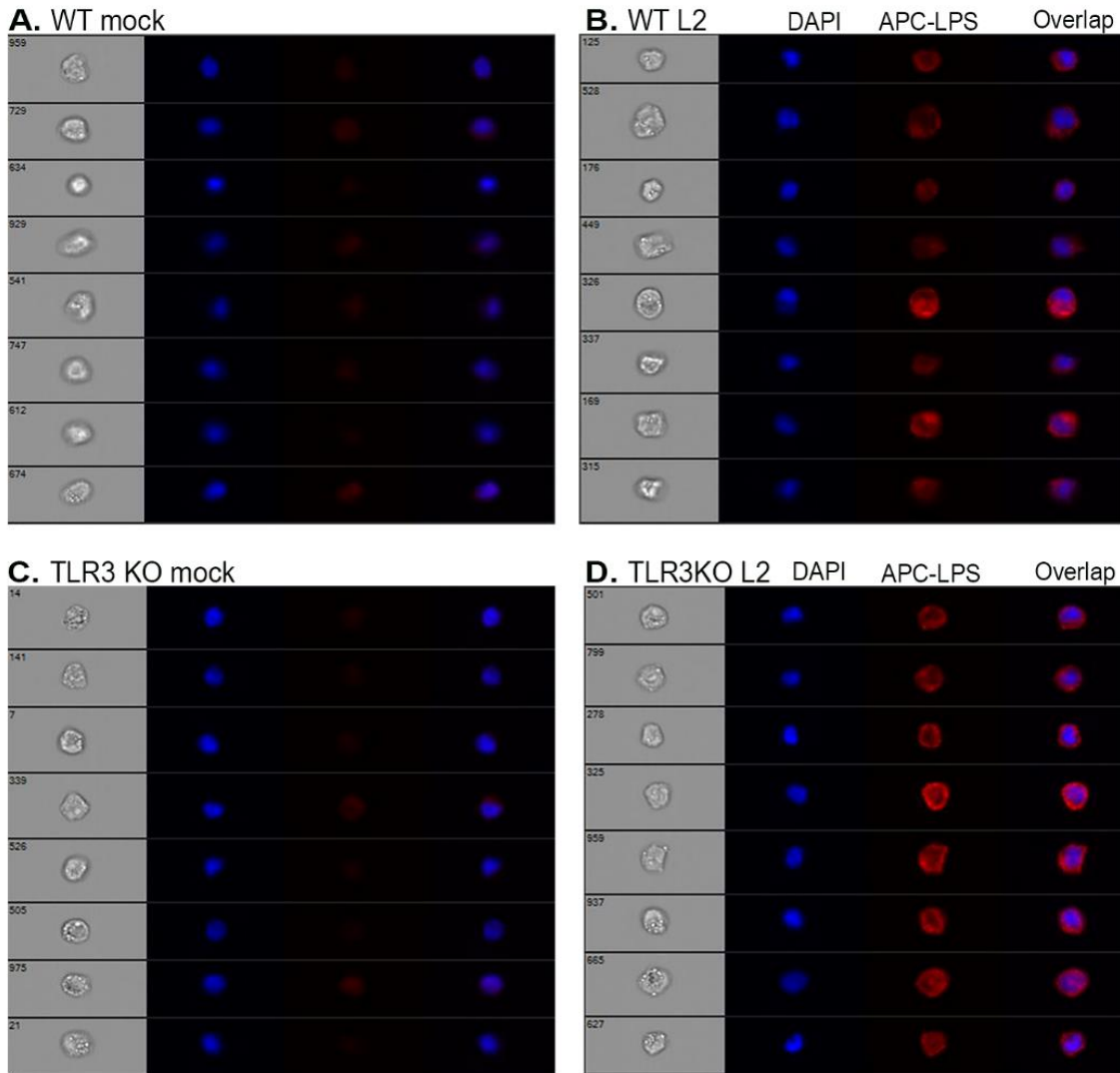
Data are representative of three independent experiments.



16

17 **FIG S3. TLR3 deficiency in murine OE cells leads to larger and aberrantly**
18 **shaped chlamydial inclusions.** WT (A) and TLR3-deficient (B) mouse OE cells were
19 infected with *C. muridarum* in triplicate at a MOI of 10 IFU/ cell for 24hrs. The chlamydial
20 inclusion was stained using anti-chlamydial LPS monoclonal antibody and detected via
21 Alexa-fluor 488 conjugated secondary antibody. *Data shown are representative. Arrows*
22 *show smaller vs. larger inclusion; magnification 60x.*

23



24

25

26

27

28

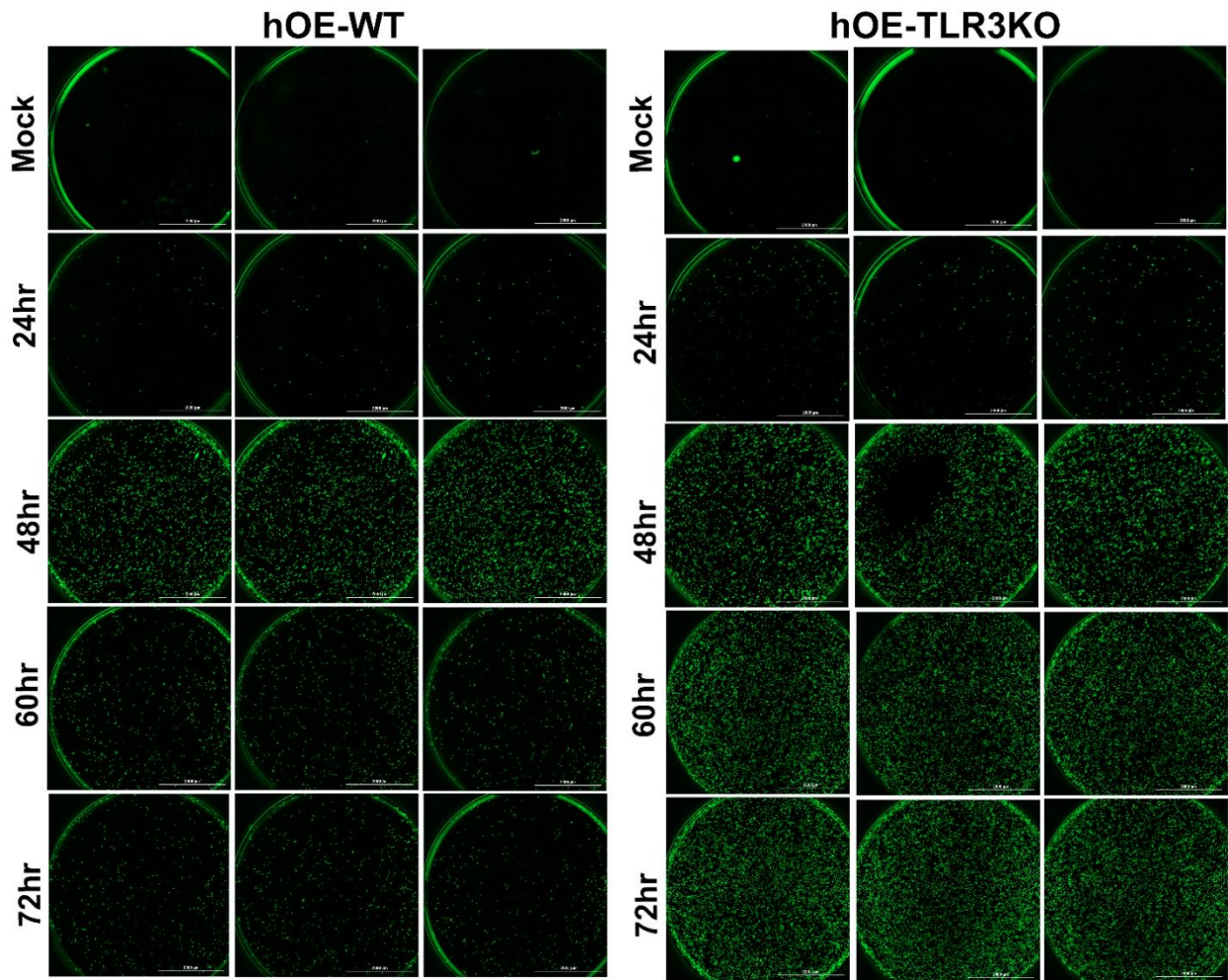
29

30

31

32

FIG S4. Image Stream X analyses of the chlamydial inclusion in WT and TLR3-deficient hOE cells. WT and TLR3-deficient (KD) hOE cells were either mock-treated or infected with *C. trachomatis* L2 in triplicate at a MOI of 10 IFU/ cell for 24hrs. The chlamydial LPS was stained using anti-*Chlamydia* LPS monoclonal antibody and detected via allophycocyanin (APC) conjugated secondary antibody, while APC-conjugated anti-IgG served as an isotype staining control. Individual cells were visualized and photographed using multi-spectral imaging flow cytometry. Nuclei were visualized via DAPI staining.



33

34

FIG S5. TLR3 deficiency has an impact on chlamydial replication. WT and

35

TLR3 deficient hOE cells were either mock treated or infected with *C. trachomatis*-serovar

36

D at MOI of 5 IFU/ cell for 72hrs. The infected cells were harvested at 24, 48, 60, and

37

72hrs post-infection, and the cell lysates were collected, sonicated, and titrated on fresh

38

Hela cell monolayers. Chlamydial inclusions were identified using anti-chlamydial LPS

39

monoclonal antibody and detected via Alexa-fluor 488 conjugated secondary antibody

40

after images were captured using the EVOS FL auto cell-imaging system. The data

41

presented are representative of three independent experiments.

42