

1 **Supplementary figure legend**

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3 ***Supplementary table 1. Sequence of sense and antisense primers use in RT-QPCR experiments.***

4

5 ***Supplementary table S2. Expression of IL-1 $\beta$  receptor mRNA.***

6

7 ***Supplementary Figure 1. Y. pseudotuberculosis infected does not increase the number of***  
8 ***apoptotic in Peyer's patches.***

9 PP from WT mice were mounted in Ussing chamber and incubated or not with *Yersinia* pIB102  
10 strain at  $1.10^7$ cfu/mL. Then, the number of apoptotic cells identified by caspase-3 immunostaining  
11 was counted inside dome and FAE from PP. 2 hours of incubation with the *Yersinia* pIB102 strain  
12 did not modify the number of apoptotic cells inside the dome and FAE of PP. Enlargements of the  
13 fields delineated by the rectangles are shown in A' and B' (Bars: 250  $\mu$ m).

14

15 ***Supplementary Figure 2. TLR-2 and/or TLR-4 activation increases IL-1 $\beta$  expression.***

16  $10^6$  THP-1 cells were infected with *Yersinia* pIB102 strain (MOI10) or stimulated with Pam4CSK3  
17 (20 $\mu$ g/mL) and/or LPS (20 $\mu$ g/mL) from *E. coli* for 2 hours. Then THP-1 cells were centrifuged and  
18 the IL-1 $\beta$  level in the supernatant was measured by ELISA. (at least n=8 per group; mean $\pm$ s.e.m;  
19 \*\*\*P<0.001 vs. THP-1 group; +++P<0.001 vs. THP-1 pIB102 infected group).

20

21 ***Supplementary Figure 3. TLR-2/4 are required for the Y. pseudotuberculosis increases TNF $\alpha$***   
22 ***secretion in THP1 cells, without modification of the permeability of the Caco-2 monolayer.***

23 (A-C) THP-1 cells were transfected with si-RNA TLR-2, TLR-4 or, with si-RNA not targeting TLR  
24 receptor (NT) and infected with *Yersinia* pIB102 strain (MOI10) for 2 hours. After centrifugation,  
25 the levels of IL-8 (A), IL-12 (B) and TNF $\alpha$  (C) were measured in the supernatant (at least n=6 per  
26 group; 3 independent experiments; mean $\pm$ s.e.m; \*P<0.05 and, \*\*\*P<0.001 vs. NT group; +P<0.05  
27 and ++P<0.01 vs. NT pIB102 group; \$\$\$P<0.001 vs. siRNA TLR-2 pIB102 group). (D) Caco-2 cells  
28 were cultivated in a Transwell system for 12 days. Following 24 hours of anti-TNF $\alpha$  (25, 50 or  
29 100 $\mu$ g/mL) treatment,  $10^6$  THP-1 cells, infected for 2 hours with *Yersinia* pIB102 strain (MOI10),  
30 were added into the basolateral compartment of Transwell chamber and paracellular permeability  
31 was monitored during 24 hours. (at least n=8 per group; 3 independent experiments; mean $\pm$ s.e.m;  
32 \*\*\*P<0.001 vs. THP-1 group).

33 ***Supplementary Figure 4. Caspase-1 inhibitor treatment does not alter the synthesis of IL-8, IL12***  
34 ***and TNF $\alpha$  by THP-1 cells in response to *Y. pseudotuberculosis*.***

35 (A-D) THP-1 cells were infected or not with the *Yersinia* pIB102 strain (MOI10) for 2 hours. To test  
36 the role of Caspase-1 in the synthesis of IL-1 $\beta$ , IL-8, IL-12 and TNF $\alpha$ , THP-1 cells were treated for  
37 24 hours with Z-YVAD-FMK (20 $\mu$ g/mL) before *Yersinia* infection. After centrifugation, the levels  
38 of IL-1 $\beta$  (A), IL-8 (B), IL-12 (C) and TNF $\alpha$  (D) were measured in the supernatant (at least n=6 per  
39 group; 3 independent experiments; mean $\pm$ s.e.m; \*\* P<0.01 and \*\*\* P<0.001 vs. THP-1 group).

40

41 ***Supplementary Figure 5. IL-1 $\beta$  produced by pIB102-infected THP-1 cells triggers a***  
42 ***condensation of actomyosin cytoskeleton adjacent to the tight junction complex of polarized***  
43 ***Caco-2 monolayer.***

44 (A-B) Caco-2 cells were cultivated into a Transwell system for 12 days. Then, 10<sup>6</sup> THP-1 cells  
45 infected or not for 2 hours with *Yersinia* pIB102 strain (MOI10) were added into the basolateral  
46 compartment of Transwell chamber. Following 24 hours of incubation, the condensation of  
47 actomyosin cytoskeleton adjacent to the apical junctional complex was investigated by electron  
48 microscopy. Enlargements of the fields delineated by the rectangles are shown in A' and B' (Bars:  
49 100 nm). Electron micrographs of Caco-2 epithelial cells demonstrate a marked condensation of  
50 actomyosin cytoskeleton (arrow) following incubation of Caco-2 monolayer with pIB102 infected  
51 THP-1 cells for 24 hours.

52

53 ***Supplementary Figure 6. Schematic illustration of the mechanisms involved in the disruption of***  
54 ***the epithelial barrier function in Peyer's patches by *Y. pseudotuberculosis*.***

55 Following adhesion of *Y. pseudotuberculosis* with the FAE of PP, *Y. pseudotuberculosis* is  
56 recognized by immune cells of PP and triggers IL-1 $\beta$  (1) synthesis by a TLR-2 dependent  
57 mechanism. TLR-2 enhances pro-IL-1 $\beta$  expression (2) and Caspase-1 activity (3) inside immune  
58 cells of the PP. Then, by interacting with IL-1 $\beta$  receptors expressed by of the Follicle associated  
59 epithelial cells, IL-1 $\beta$  stimulates expression and activity of the MLCK (5) in a NF $\kappa$ B (4) dependant  
60 manner. The increase of the MLCK activity in turn increases the paracellular and transcellular  
61 permeability (6) of the follicle associated epithelium.

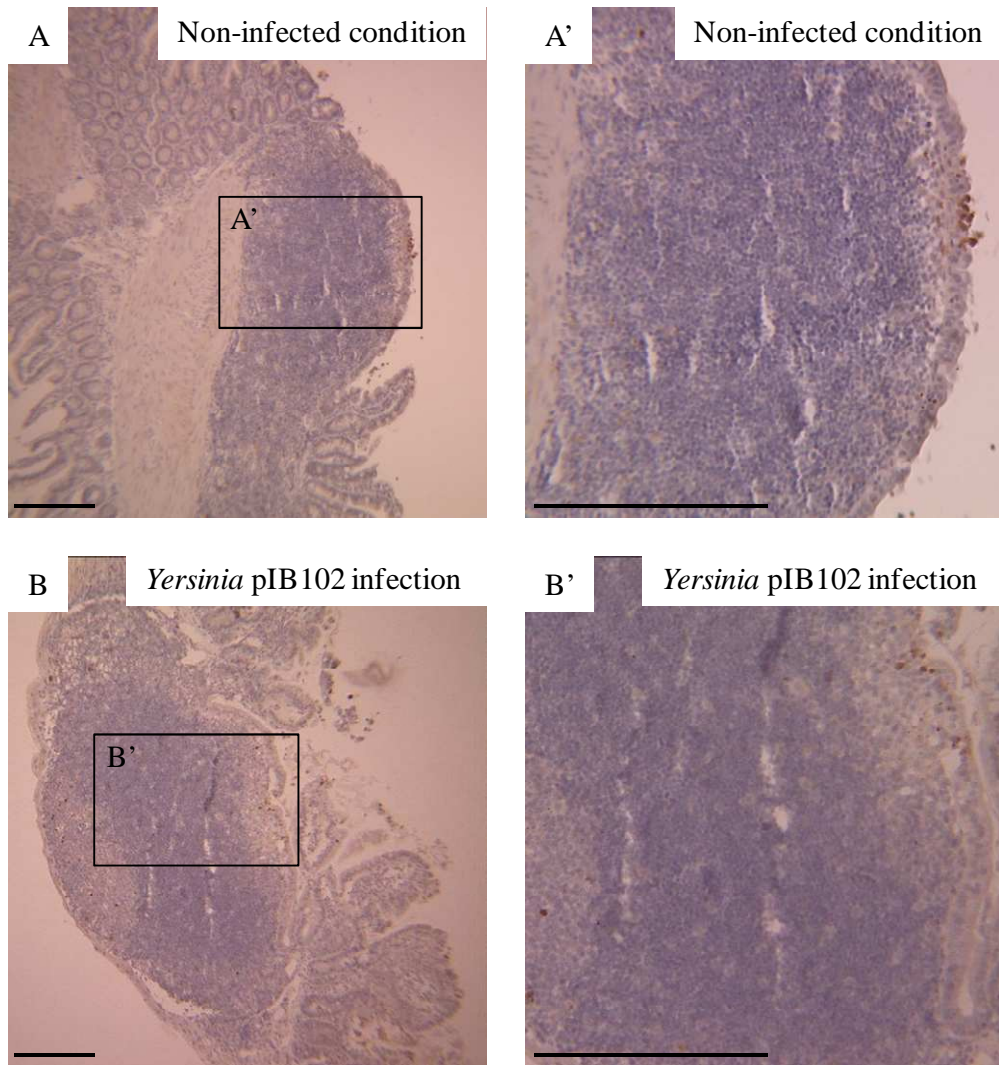
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63 ***Supplementary Figure 7. Reconstitution of chimeric mice.***

64 Twelve weeks after bone marrow reconstitution of mice, the chimerism for TLR-2 expression was  
65 monitored inside spleen and PP. (A) The percentages of CD45.Ly.1 or Ly.2 positive immune cells  
66 inside spleen and PP were analyzed by flux cytometer. (B-E). The percentage of positive immune  
67 cells for CD3, CD19 and CD11c reconstitution were analyzed by flux cytometer. (B and C)  
68 represents the levels of *TLR-2*<sup>+/+</sup> (Ly5.1 marker) reconstitution inside spleen (B) and PP (C) from  
69 the irradiated *TLR-2*<sup>-/-</sup> mice (Ly5.2 marker). (D and E) represents the levels of *TLR-2*<sup>-/-</sup> mice (Ly.5.2  
70 marker) reconstitution inside spleen (D) and PP (E) from the irradiated *TLR-2*<sup>+/+</sup> mice (Ly5.1  
71 marker). (at least n=5 per group; mean±s.e.m).

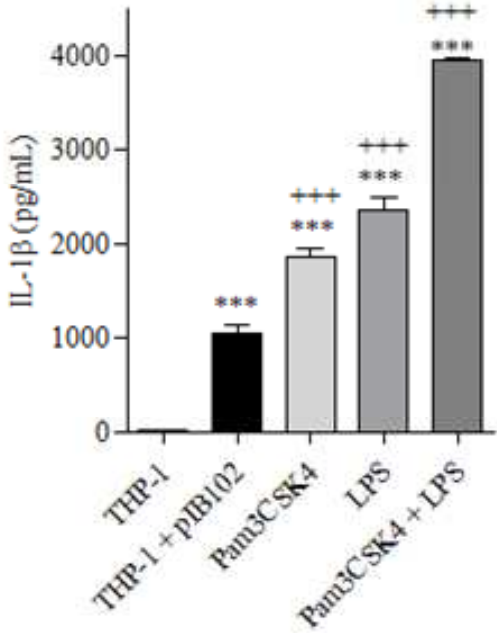
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Supplementary Figure 1.



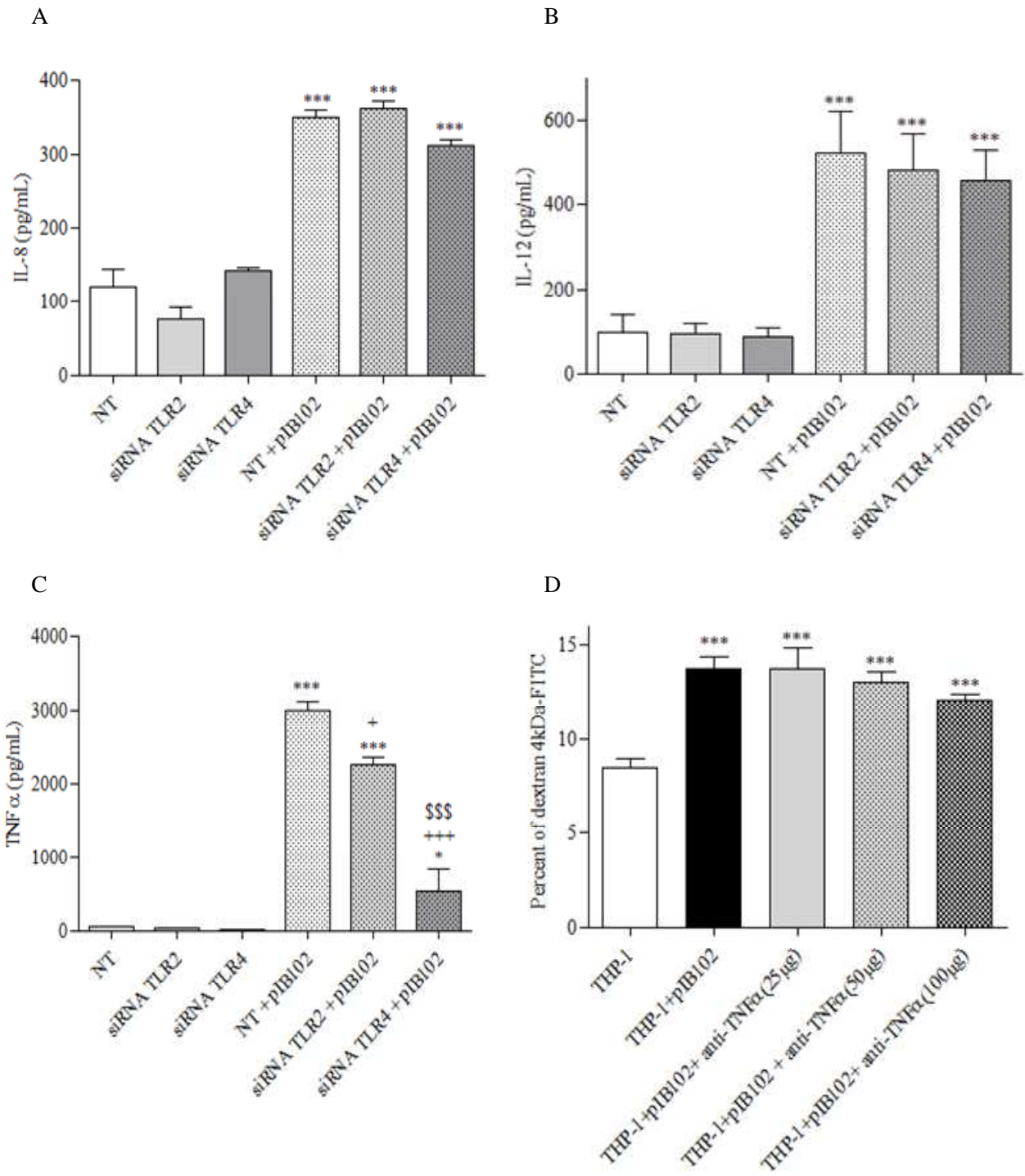
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Supplementary Figure 2.



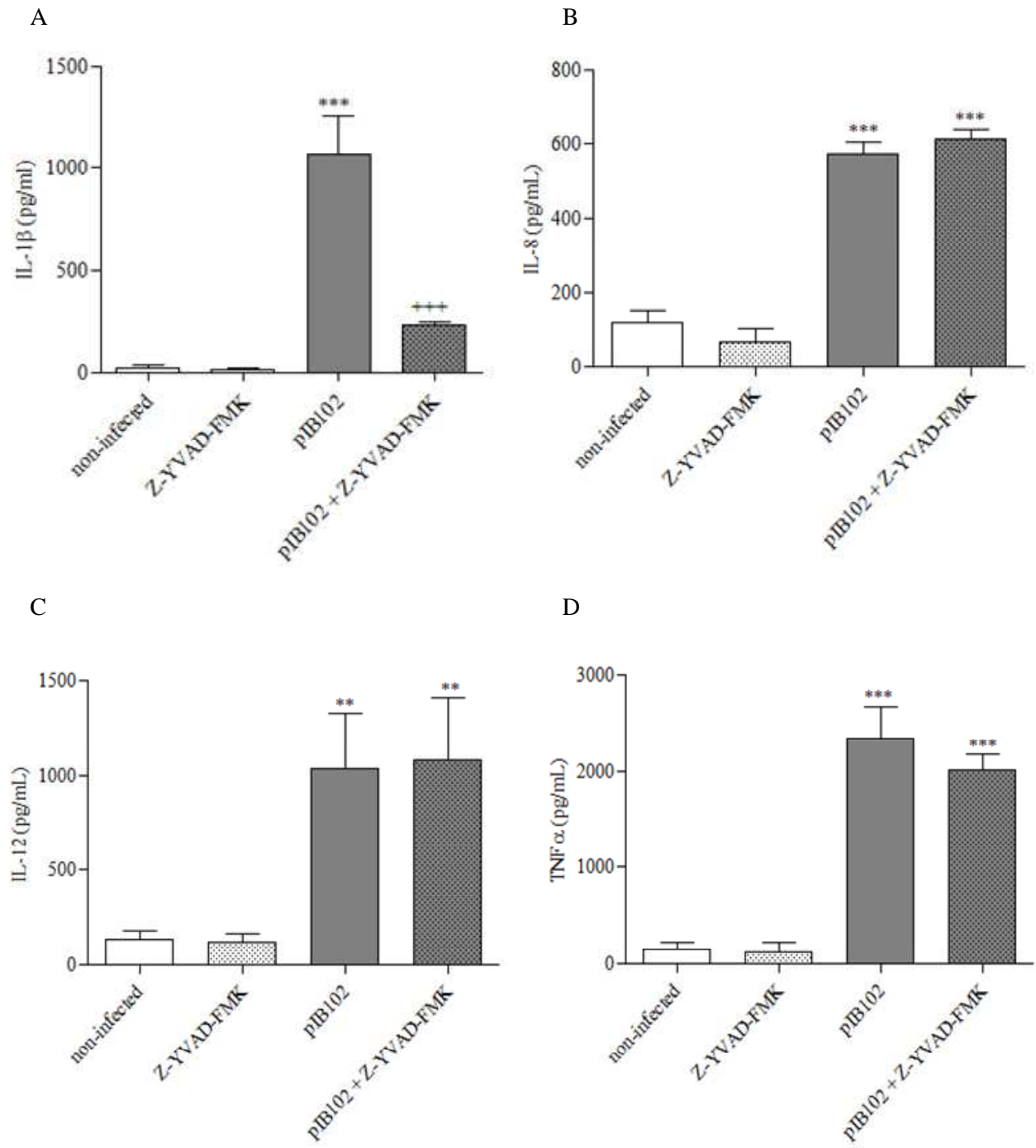
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Supplementary Figure 3.



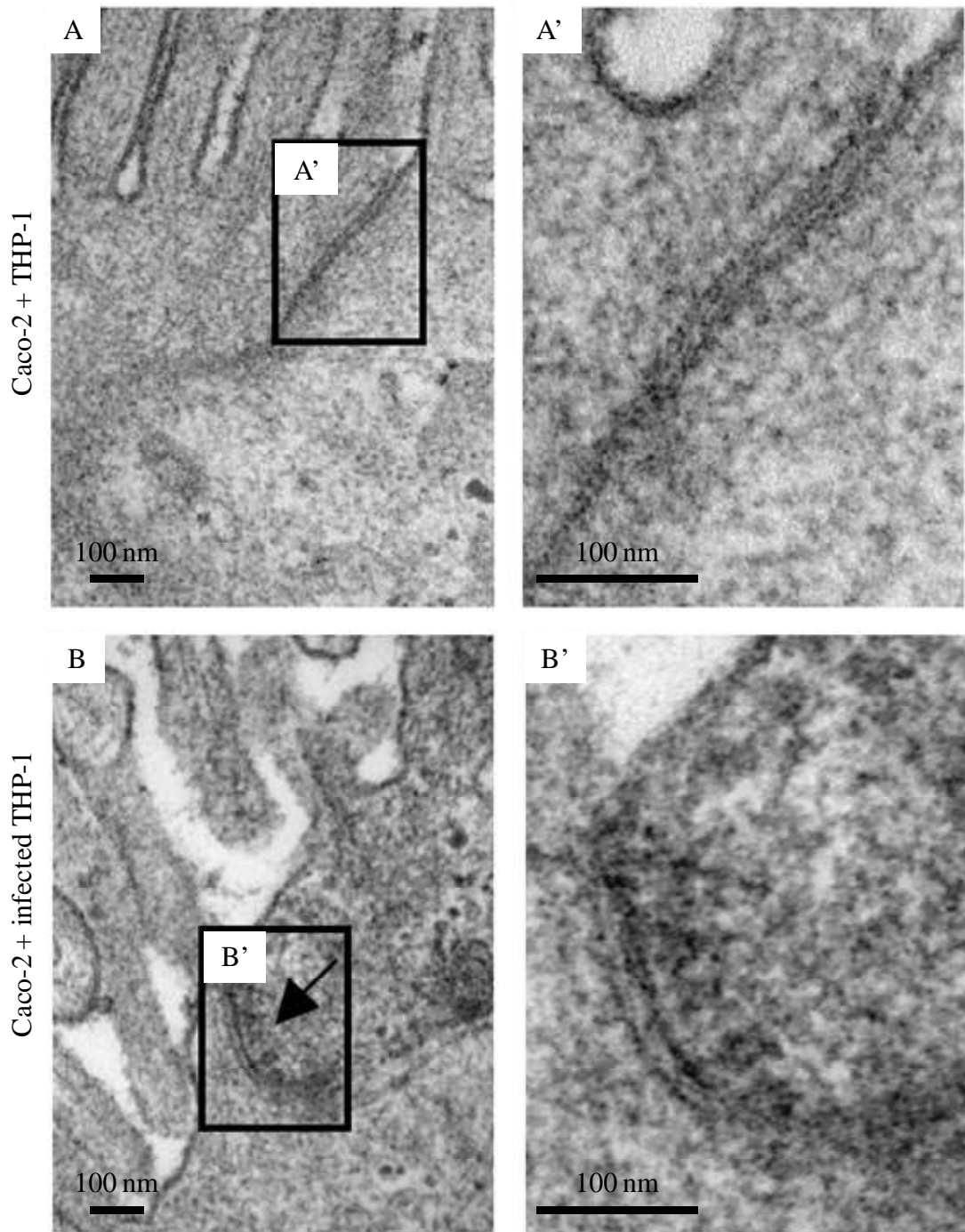
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Supplementary Figure 4.



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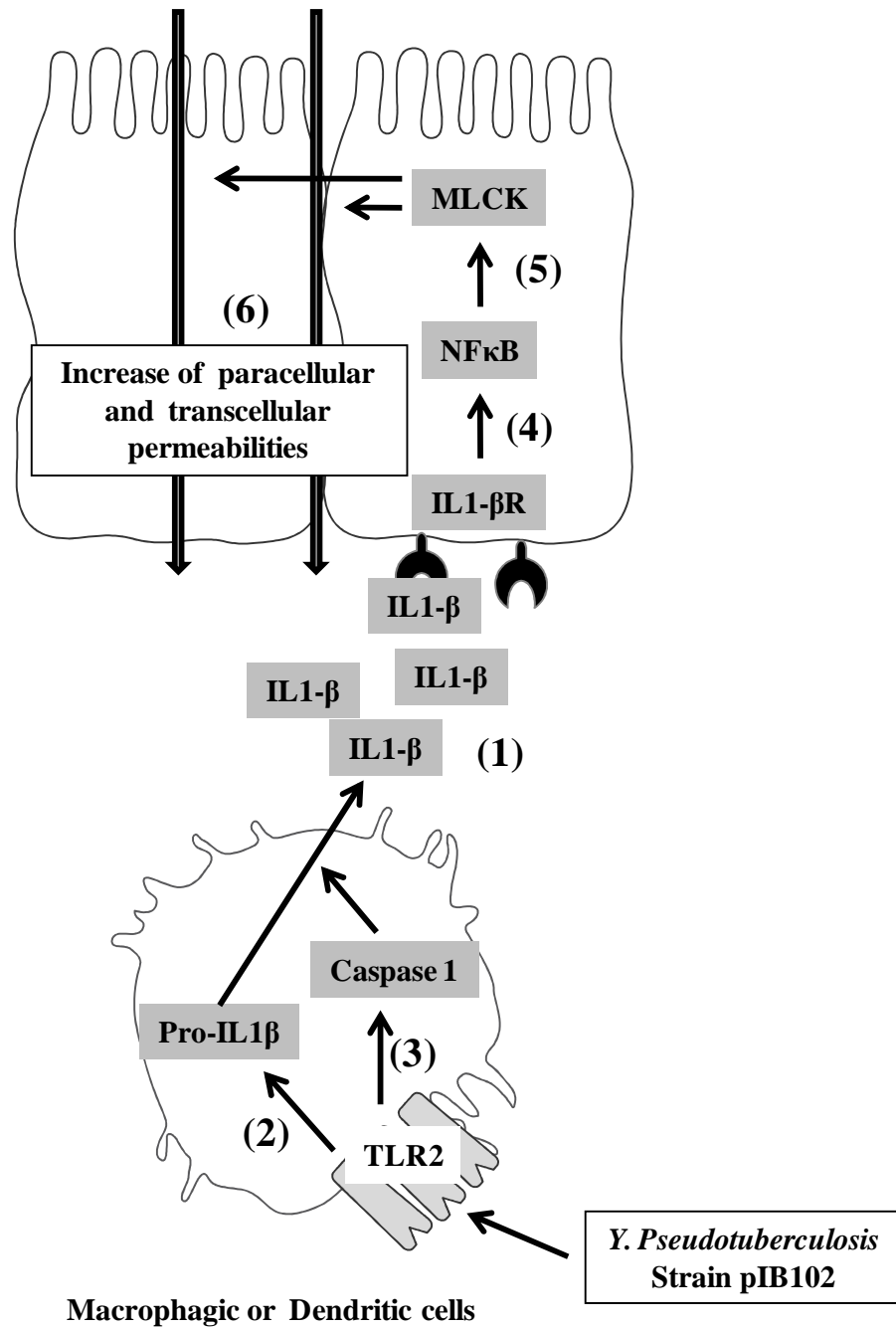
Supplementary Figure 5.



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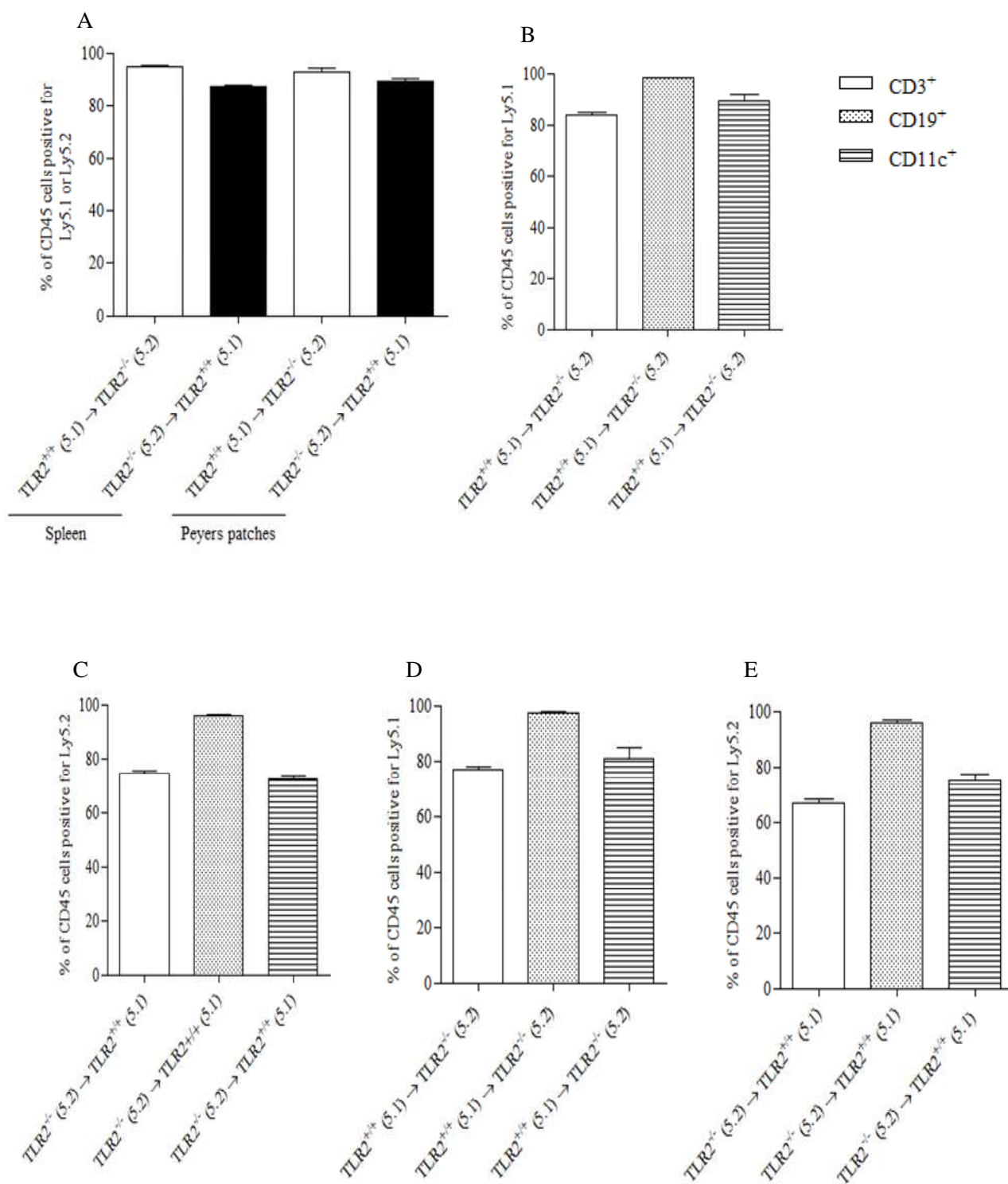
Supplementary figure 6.



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Supplementary figure 7.



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**Supplementary Table S1. Sequence of sense and antisense primers used in RT-PCR experiments.**

	Sense	Antisense
<b>Mouse</b>		
G3PDH	5'-CACCATCTTCCAGG AGCGAG-3'	5'-GCCTTCTCCATGGTG GTGAA-3'
long MLCK isoform	5'-ACATGCTACTGAGT GGCCTCTCT-3'	5'-GGCAGACAGGACATT GTTTAAGG-3'
<b>Human</b>		
G3PDH	5'-AGCAATGCC TCC TGC ACC A-3'	5'-CAGTCTTCTGGGTGG CAGTGA-3'
long MLCK isoform	5'-TGGTTGCCTCGTC AC ACATTT-3'	5'-ACCCGCCCTTCGAAC TTG-3'
TLR-2	5'-TTTGTTCCTTACAGT GAG CGG G-3'	5'-GACCATAAGGTTCTC CAC CCAG-3'
TLR-4	5'-CCC TGC GTG GAG GTG GT-3'	5'-TTT TGT AGA AAT TCA GCT CCA TGC-3'

**Supplementary Table S2. Expression of IL-1 $\beta$  receptor mRNA.**

	Caco-2 Non-infected THP-1	Caco-2 infected THP-1
IL-1 $\beta$ receptor	1.00 $\pm$ 0.04	0.90 $\pm$ 0.07

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88

89 **Supplementary Material and Methods.**

90 ***Immunohistochemistry of apoptotic cell (Caspase-3 staining).***

91 Peyer's patches (PP) from C57BL/6 Wild type mice were mounted in Ussing chamber and  
92 incubated with *Yersinia* pIB102 strain ( $1.10^7$ cfu/mL). Following 2 hours of incubation, PP samples  
93 were fixed in 4% phosphate buffered formalin and embedded in paraffin blocks and cut into 5 $\mu$ m  
94 sections. PP sections were deparaffinied and stained using the ABC standart method. Briefly, 5  $\mu$ m  
95 deparaffinised sections were subjected to a heat-induced antigen recovery in sodium citrate buffer  
96 solution pH6. Endogen peroxydase was blocked with 3% H<sub>2</sub>O<sub>2</sub> (DAKO, Carpinteria, CA, USA) and  
97 slides were incubated for 30 min with primary antibodies (rabbit polyclonal antibody against  
98 Cleaved Caspase-3 (Asp 175, dilution: 1/100, Cell Signaling Technology, Inc Ozyme, Beverly, MA,  
99 USA). A biotin-labeled secondary antibody was then applied for 30 min, followed by avidin-biotin-  
100 peroxydase conjugate for 30 min (Vector Laboratories, Burlingame, CA, USA). For detection,  
101 peroxidase enzyme substrat 3,3'-diaminobenzidine was added to yield a brown reaction product.

102 ***IL-1 $\beta$ , IL-8, IL-12 and TNF $\alpha$  production by THP-1.***

103 THP-1 cells were infected with *Yersinia* pIB102 strain (MOI10). Following 2 hours of  
104 incubation, *Yersinia* was killed by adding gentamycin (100 $\mu$ g/mL) into the medium. Then, cell  
105 suspensions were centrifuged and, IL-1 $\beta$ , IL-8, IL-12 and TNF $\alpha$  levels in the supernatants were  
106 measured by ELISA (BD biosciences), according the according to the manufacturer's instructions.

107 ***Preparation of cell suspensions from Peyer's Patches and spleen.***

108 Briefly, Peyer's patches and spleen were removed from chimeric mice and washed with cold  
109 PBS. Cell suspensions from Peyer's patches and spleen were prepared by manually extracting the  
110 cells using a 5-ml polypropylene syringe piston. The cells were centrifuged, washed in PBS and  
111 erythrocytes were lysed by addition of Gey's-solution. Then, cells from PP and spleen were  
112 suspended in 2 ml of PBS.

113 ***Flow cytometry analysis.***

114 Cell suspensions ( $10^5$ ) were incubated with PE-, FITC-, APC, or PerCP-conjugated mAbs  
115 against mouse CD3, CD11c and CD19 CD45Ly.1 and CD45Ly.2 (BD Biosciences) at optimal  
116 concentrations for 20 minutes at 4°C. Labeled cells were analyzed with a BD-LSR II apparatus and  
117 CELLQuest software (BD Biosciences).

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