Stimulation of Toll-Like Receptors profoundly influences the titer of polyreactive antibodies in the circulation

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Legend for Supplemental Figures

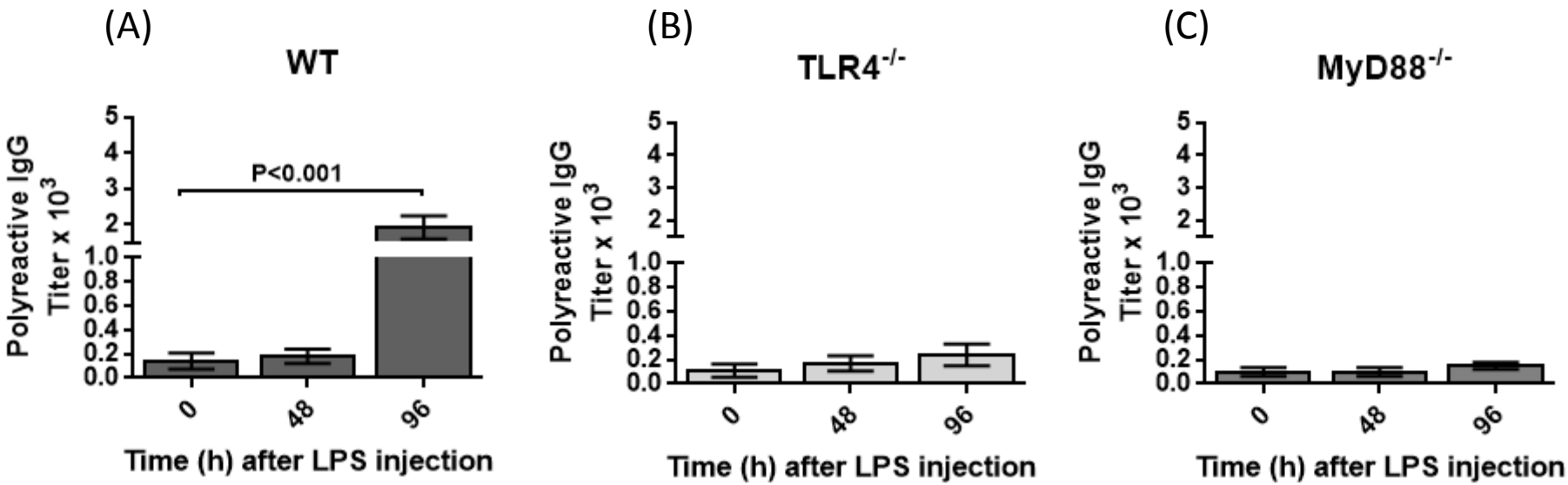
Suppl. Fig. 1. LPS stimulates the secretion of polyreactive IgG antibodies: Two months old (A) C57BL6, (B) TLR4$^{-/-}$ and (C) MyD88$^{-/-}$ mice were injected with LPS and titer of polyreactive IgG antibody was determined by ELISA on the same sera as used in Fig.1. Mean ±SEM (n=5 mice per group, ANOVA) from two independent experiments.

Suppl. Fig.2. Eluted polyreactive antibodies: Purified mouse IgM was loaded on to (A) DNP columns or (B) BSA control columns and the percentage that bound was calculated. Equal amounts of IgM from the eluted fractions then were tested for reactivity with different antigens. Data represents Mean +/- SEM of two independent experiments (ANOVA).

Suppl.Fig.3. Surrogate assay for measuring polyreactive antibody titer: (A) ELISA plates were coated with different antigens and polyreactive antibody titer was determined by serial two fold dilutions. Dotted line represents plate background activity. (B) Polyreactive antibody titer with different antigens. Data represents Mean ±SEM (n=20 mice).

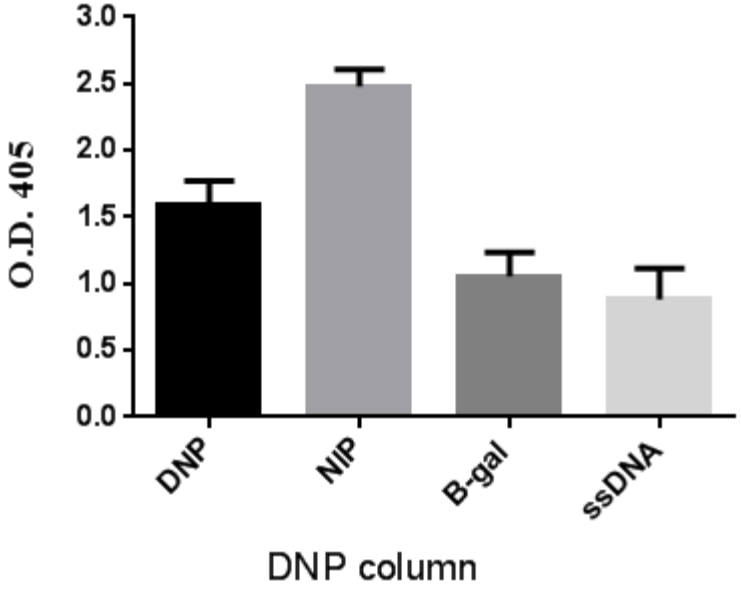
Suppl.Fig.4. LPS triggers polyreactive antibody secretion. The polyreactive antibody titer in sera after injection of mice with LPS. Elisa plates were coated with (A) Beta-galactosidase, (B) single stranded DNA and (C) NIP. Data represents Mean +/- SEM of (n=5 mice per group) two independent experiments (ANOVA).

Suppl. Fig. 1.

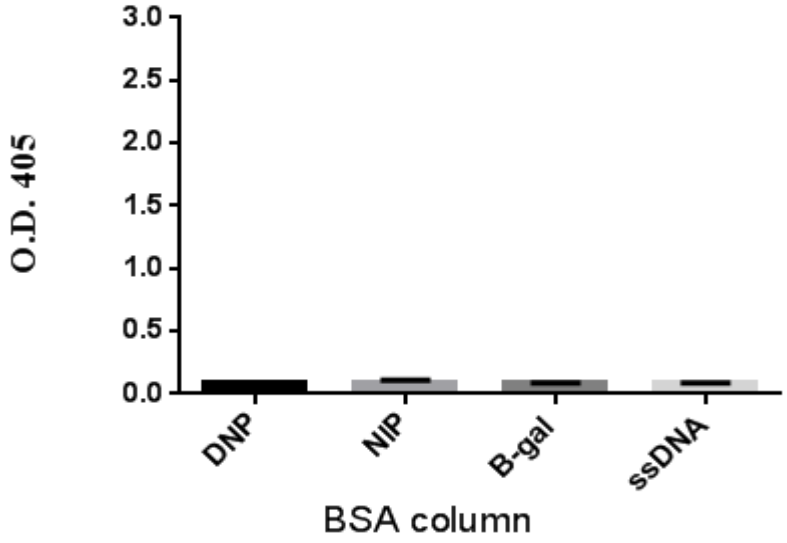

(A)
WT
P<0.001
Polyreactive IgG
Titer x 10^3
5
4
3
2
1.0
0.8
0.6
0.4
0.2
0.0
0
48
96
Time (h) after LPS injection
(B)
TLR4-/-
Polyreactive IgG
Titer x 10^3
Time (h) after LPS injection
(C)
MyD88-/-
Polyreactive IgG
Titer x 10^3
Time (h) after LPS injection

Suppl. Fig. 2.

(A)

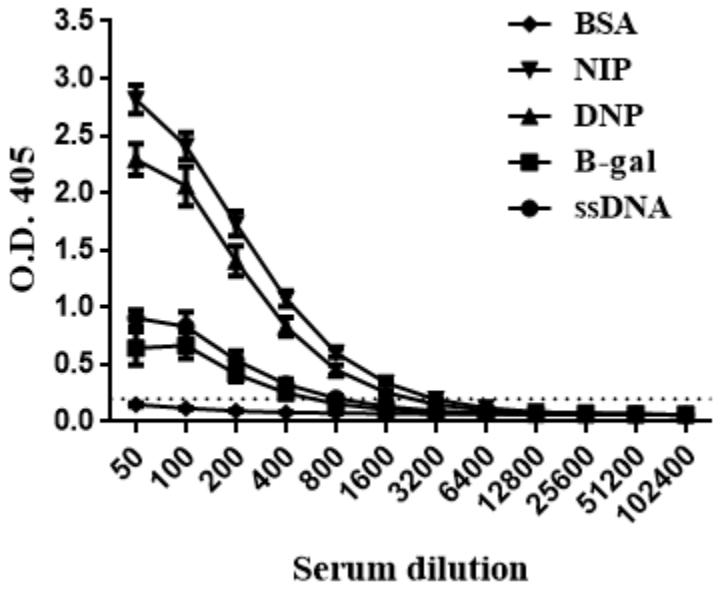


(B)

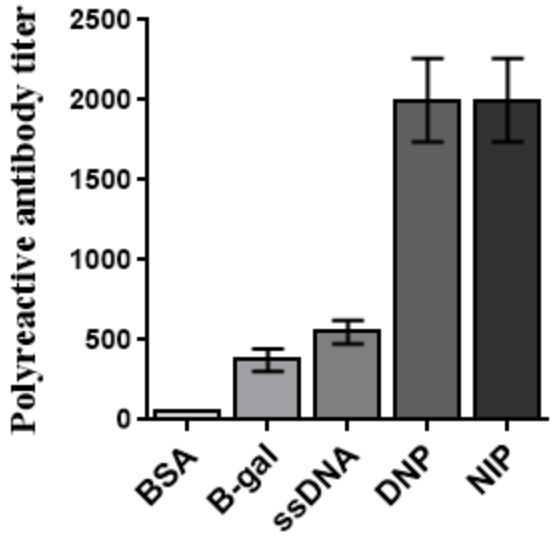


Suppl. Fig. 3.

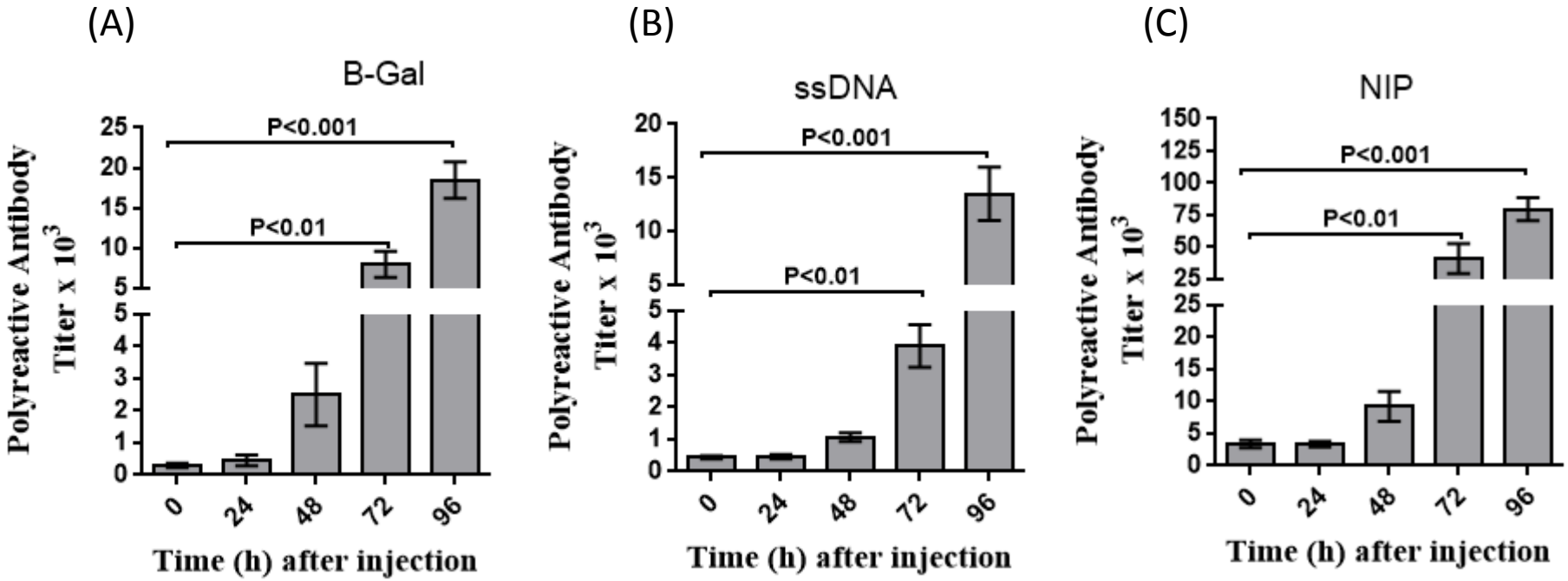
(A)



(B)



Suppl. Fig. 4.


(A)
B-Gal
P<0.001
P<0.01
Polyreactive Antibody Titer x 10^3
Time (h) after injection
(B)
ssDNA
P<0.001
P<0.01
Polyreactive Antibody Titer x 10^3
Time (h) after injection
(C)
NIP
P<0.001
P<0.01
Polyreactive Antibody Titer x 10^3
Time (h) after injection