SUPPLEMENTAL INFORMATION

Gut microbiota-induced immunoglobulin G controls systemic infection by symbiotic bacteria and pathogens

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Supplemental Figure 1

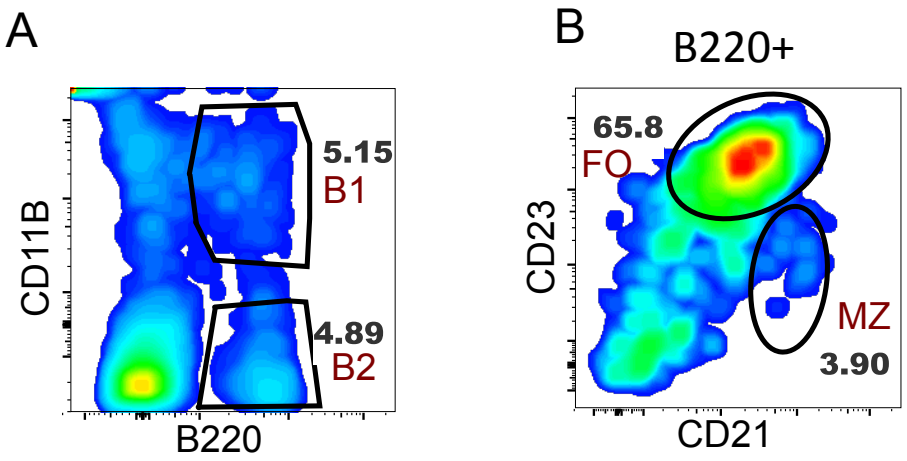

Figure S1, related to Figure 1. Gut microbiota induces antigen-specific IgG response in the steady state. Gating strategies to isolate B cells. A. B1 cells (CD11B low B220+) and B2 cells (CD11B-B220+) were isolated from freshly harvested peritoneal cells (DAPI-) from 10-12 week-old naïve WT B6 mice by FACS sorting. **B.** Splenic marginal zone (MZ) B cells (B220+CD21+CD23 low) and follicular (FO) B cells (B220+CD21lowCD23high) were isolated from freshly harvested splenocytes (DAPI-) from 10-12 week-old naïve WT B6 mice by FACS sorting.

Supplemental Figure 2

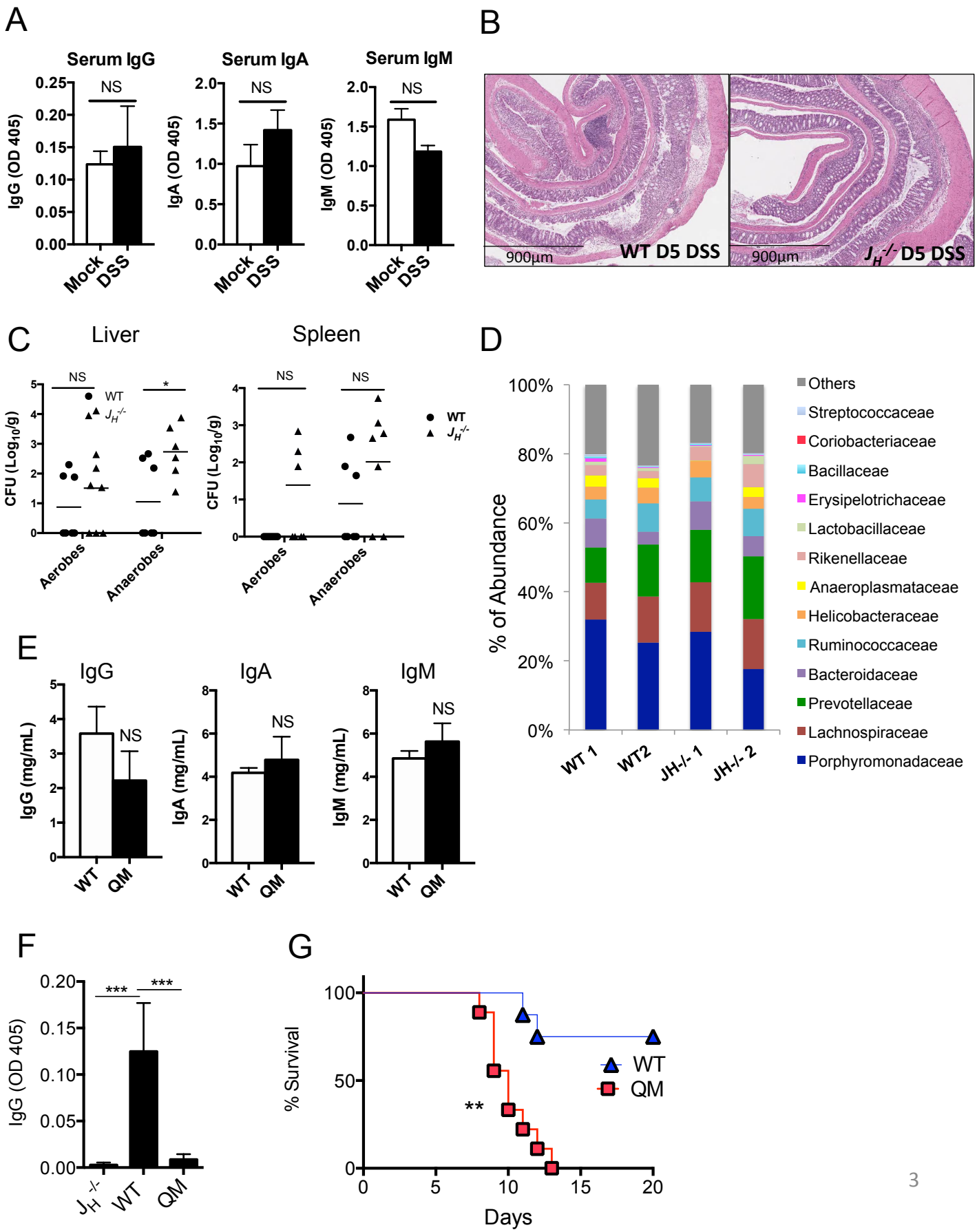

Figure S2, related to Figure 3. Gut microbiota-induced IgG confers protection against DSS-induced bacteremia

A. Serum IgG, IgA, and IgM against fecal bacteria in mock or D7 DSS-treated mice. **B.** H&E histological staining of colons from day 5 mockl or DSS-treated mice. **C.** CFU of aerobes and anaerobes in the blood, spleens and livers of WT and $J_H^{-/-}$ mice that were treated with or without 2.5% DSS for 7 days. **D.** Taxonomic compositions of fecal bacteria in age and sex-matched WT and $J_H^{-/-}$ mice after 4 weeks of co-housing. **E.** Total amounts of serum IgG, IgA and IgM in QM mice. **F.** Amounts of serum IgG in naïve $J_H^{-/-}$, WT and QM mice against fecal bacteria. **G.** Survival of WT and QM mice after administration of 2.5 % DSS in drinking water for 7 days (WT n=8; QM n=9). Data represent 2-3 independent experiments. Error bars indicate S.D. *= $p<0.05$, **=$p<0.01$, ***=$p<0.001$

Supplemental Figure 3

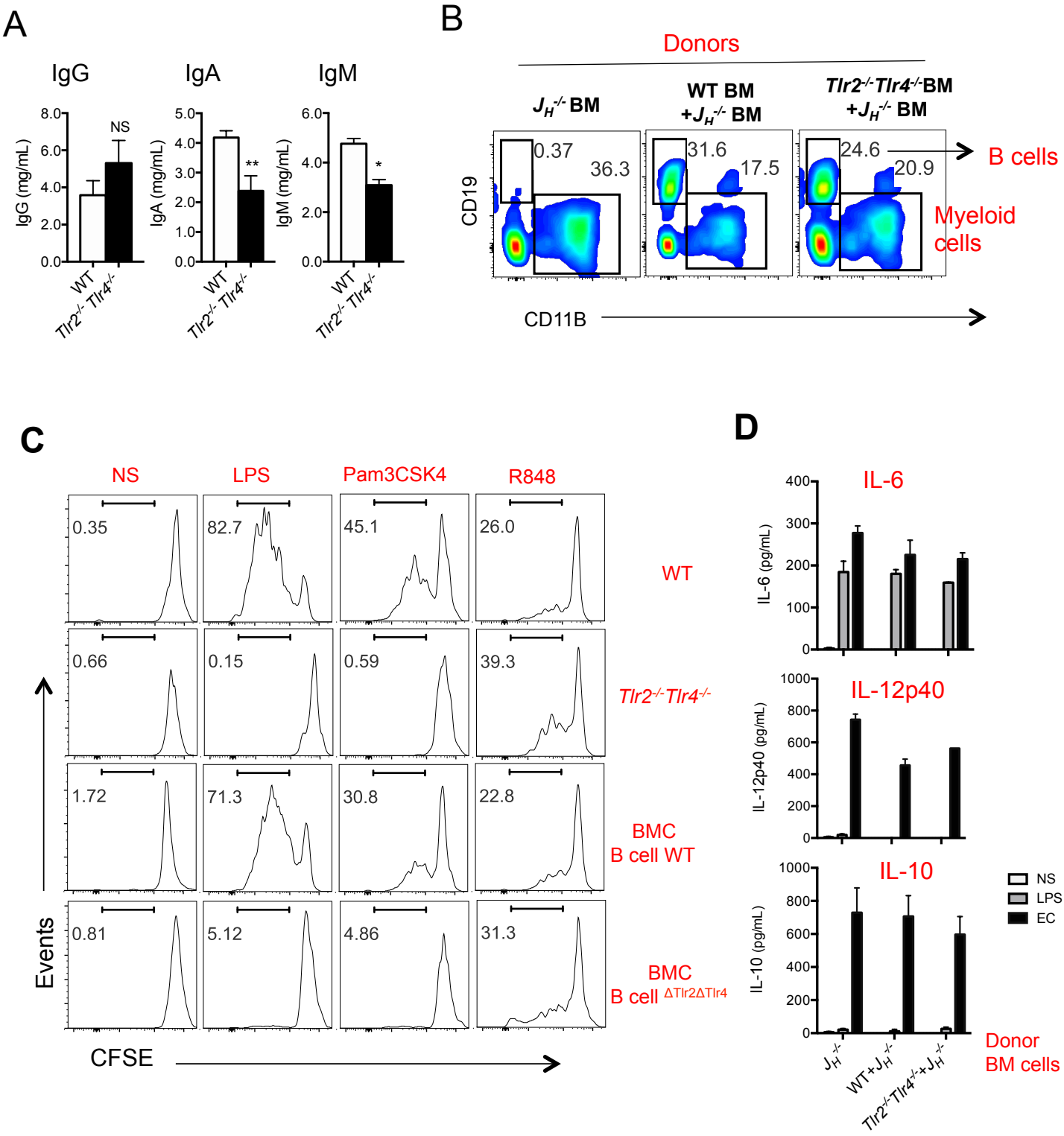

Figure S3, related to Figure 4. TLR4 signaling is required for induction of microbiota-specific IgG

A. Total serum amounts of IgG, IgA and IgM in age-matched and co-housed WT and *Tlr2*$^{-/-}$ *Tlr4*$^{-/-}$ mice (n =8-10 per genotype). **B.** Verification of B cell population (CD19+) and myeloid cell population (CD11B+CD19-) in bone marrow chimeras. Data represent 3 mice per group. **C.** Isolated from WT or *Tlr2*$^{-/-}$*Tlr4*$^{-/-}$ mice or bone marrow chimeras transplanted with WT and $J_H^{-/-}$ BM cells orWT and *Tlr2*$^{-/-}$*Tlr4*$^{-/-}$ BM cells, splenic B cells were labeled with CFSE and stimulated ex vivo with LPS, Pam3CSK4, R848 or without stimulation (NS) for 72hrs before analysis of proliferation by flow cytometry. Data are representative of 3 individual mice per group. **D.** BM macrophages were isolated by FACS sorting from WT and *Tlr2*$^{-/-}$*Tlr4*$^{-/-}$ mice or bone marrow chimeras transplanted with WT and $J_H^{-/-}$ BM cells or WT and *Tlr2*$^{-/-}$*Tlr4*$^{-/-}$ BM cells, and then stimulated ex vivo with LPS or *E. coli* for 18hrs. Amounts of IL-6, IL-12p40 and IL-10 in culture supernatants were quantified by ELISA. Error bars indicate S.D. *= $p<0.05$, **=$p<0.01$

Supplemental Figure 4

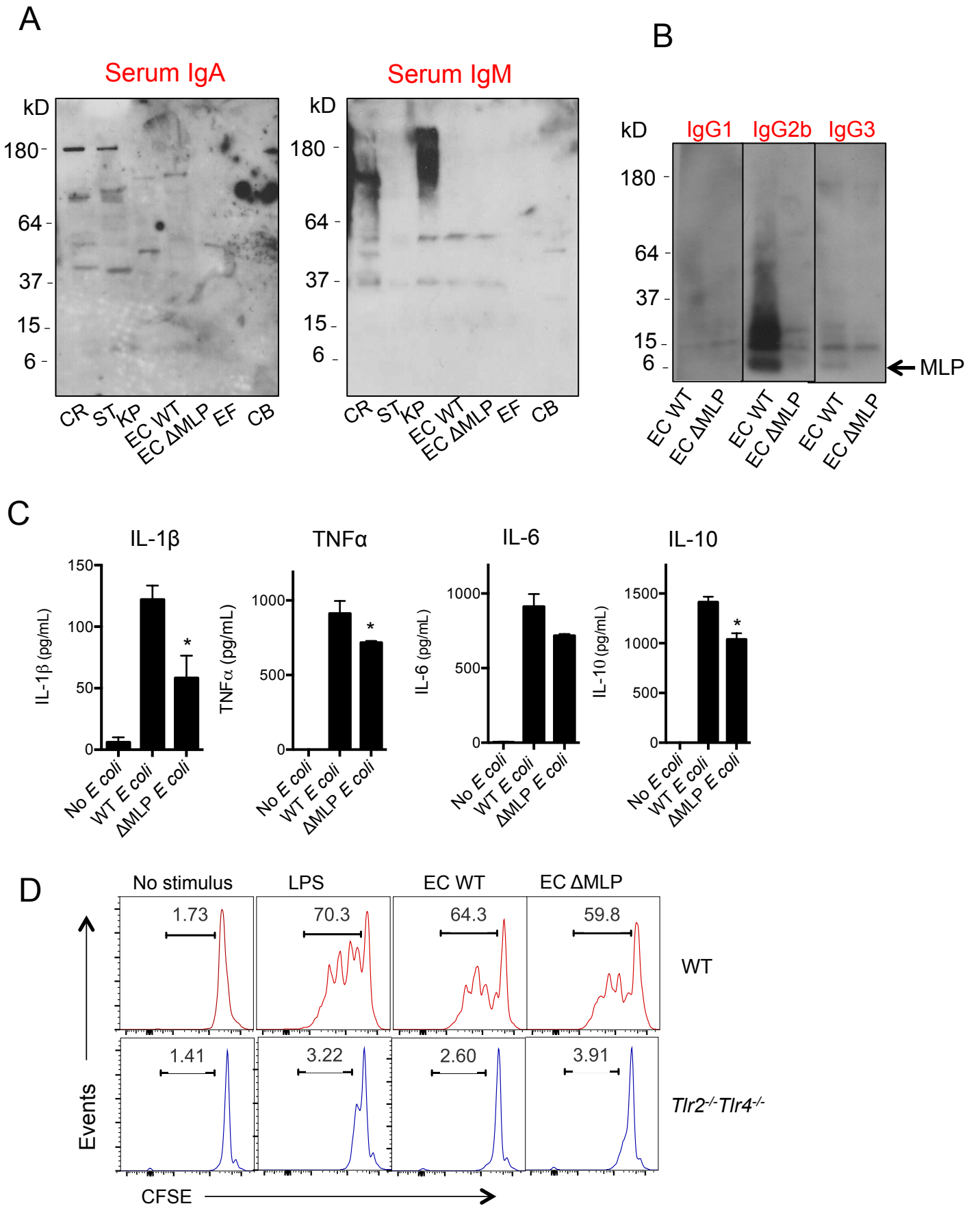

Figure S4, related to Figure 5. Gram-negative murein lipoprotein is a major microbiota-derived antigen to induce steady-state IgG response

A. Immunoblotting for IgA and IgM in the serum from a 6-week-old WT SPF mouse that bound to bacterial antigens from *Citrobacter rodentium* (CR), *Salmonella typhimurium* (ST), WT and ΔMLP *Escherichia coli* (EC), *Klebsiella pneumoniae* (KP), *Enterococcus faecalis* (EF), and *Clostridium bifermentans* (CB). **B.** Immunoblotting for IgG1, IgG2b and IgG3 in the serum of a 6-week-old WT SPF mouse that bound to bacterial proteins from WT and ΔMLP *E. coli*. **C.** BMDCs were stimulated with heat-killed WT and ΔMLP *E. coli* for 18h and the amounts of IL-1β, TNFα, IL-6 and IL-10 were measured by ELISA. **D.** CFSE labeled WT or *Tlr2*$^{-/-}$*Tlr4*$^{-/-}$ splenic B cells were stimulated with LPS, or heat-killed WT or ΔMLP *E. coli* for 72 hrs before analysis for proliferation by flow cytometry. Error bars indicate S.D. *= $p<0.05$.

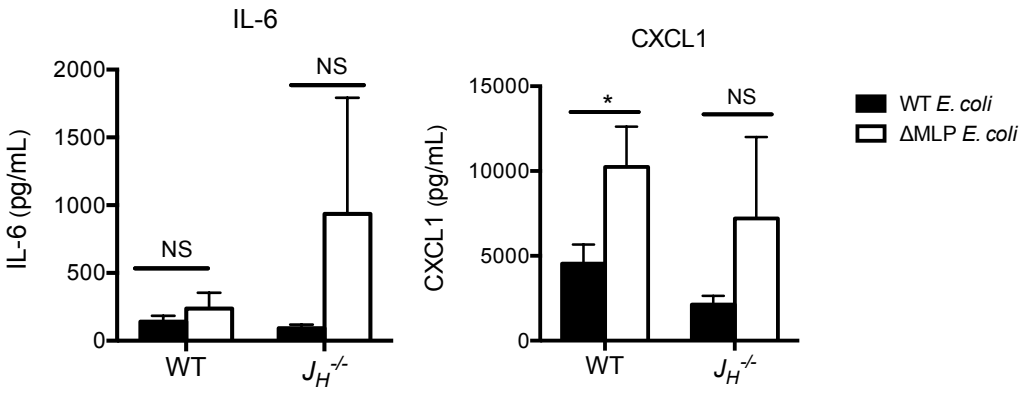

Figure S5, related to Figure 6. Anti-MLP IgG promotes killing of *E. coli* in vitro and in vivo
Serum levels of IL-6 and CXCL1 in WT mice that were i.p. infected with $5x10^7$ cfu of WT or ΔMLP *E. coli* for 24 hrs. Data represent 2-3 independent experiments. Error bars indicate S.D. *= p<0.05, **=p<0.01, ***=p<0.001

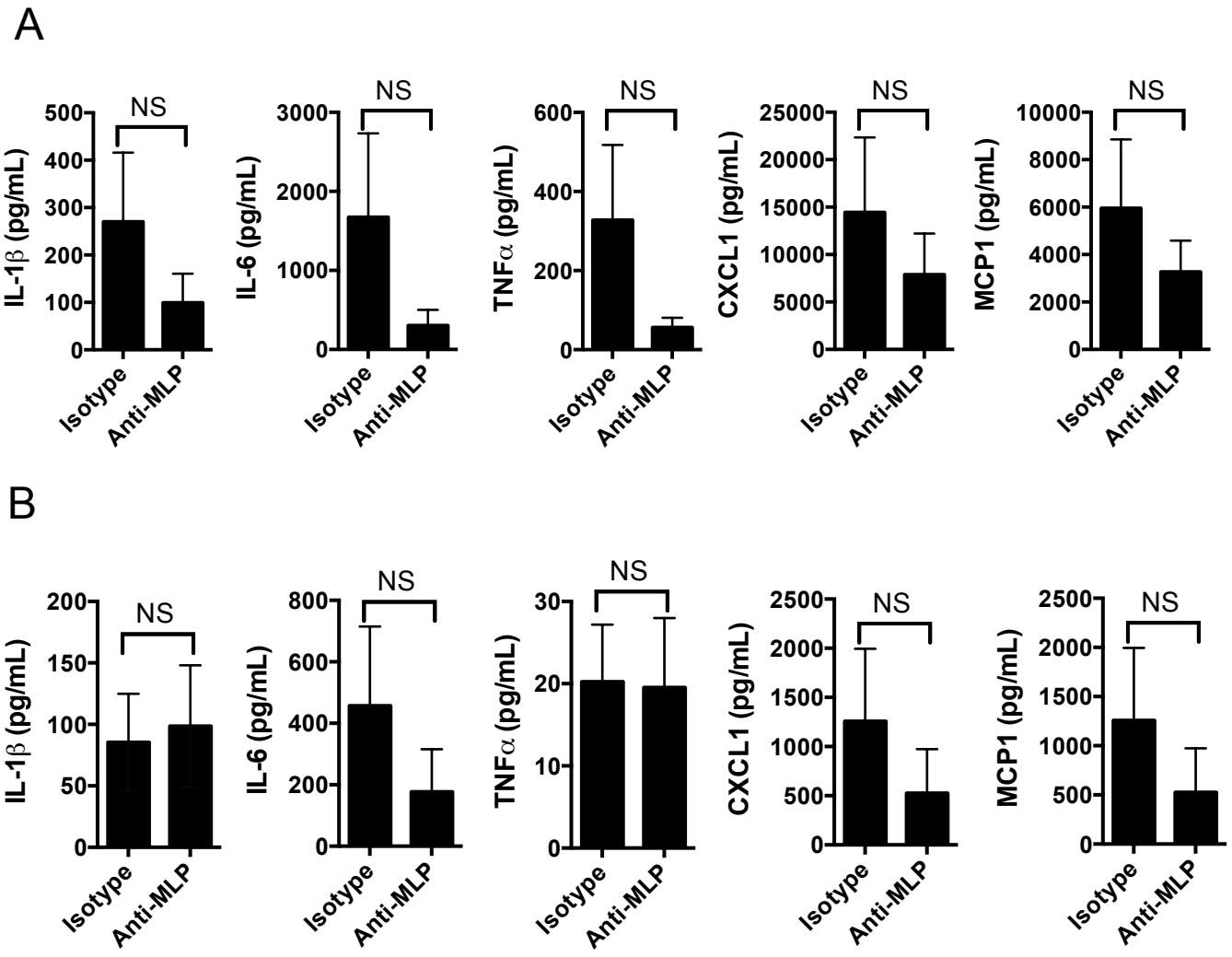

Figure S6, related to Figure 7. IgG against murein lipoprotein confers protection against *Salmonella*

A and B. Amounts of IL-1β, IL-6, TNFα, CXCL1 and MCP1 in day 3 peritoneal fluids (A) and sera (B) of 6-8 week-old WT mice that were i.p. administered 1mg of anti-MLP IgG or isotype 24 hrs before i.p. infection with $4x10^4$ cfu of ST M525P. Data represent 2 independent experiments. Error bars indicate S.D. *= $p<0.05$, **=$p<0.01$, ***=$p<0.001$