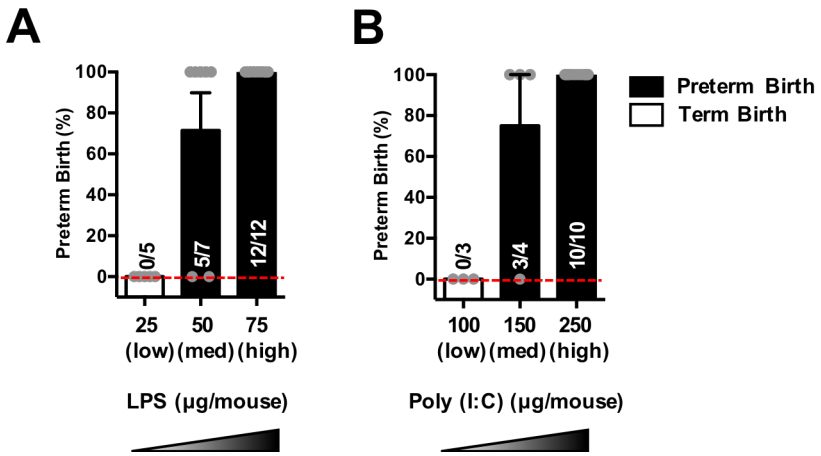
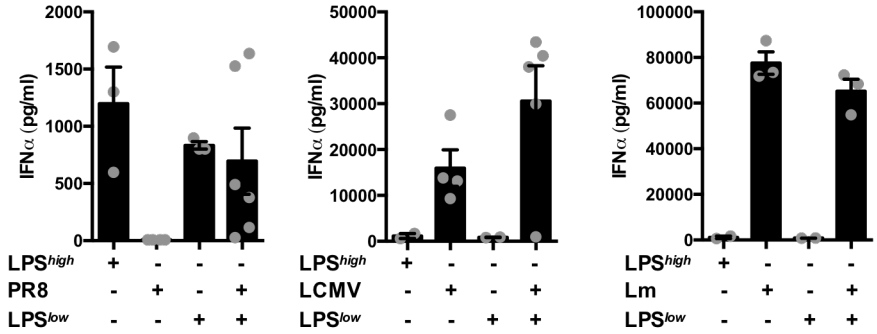
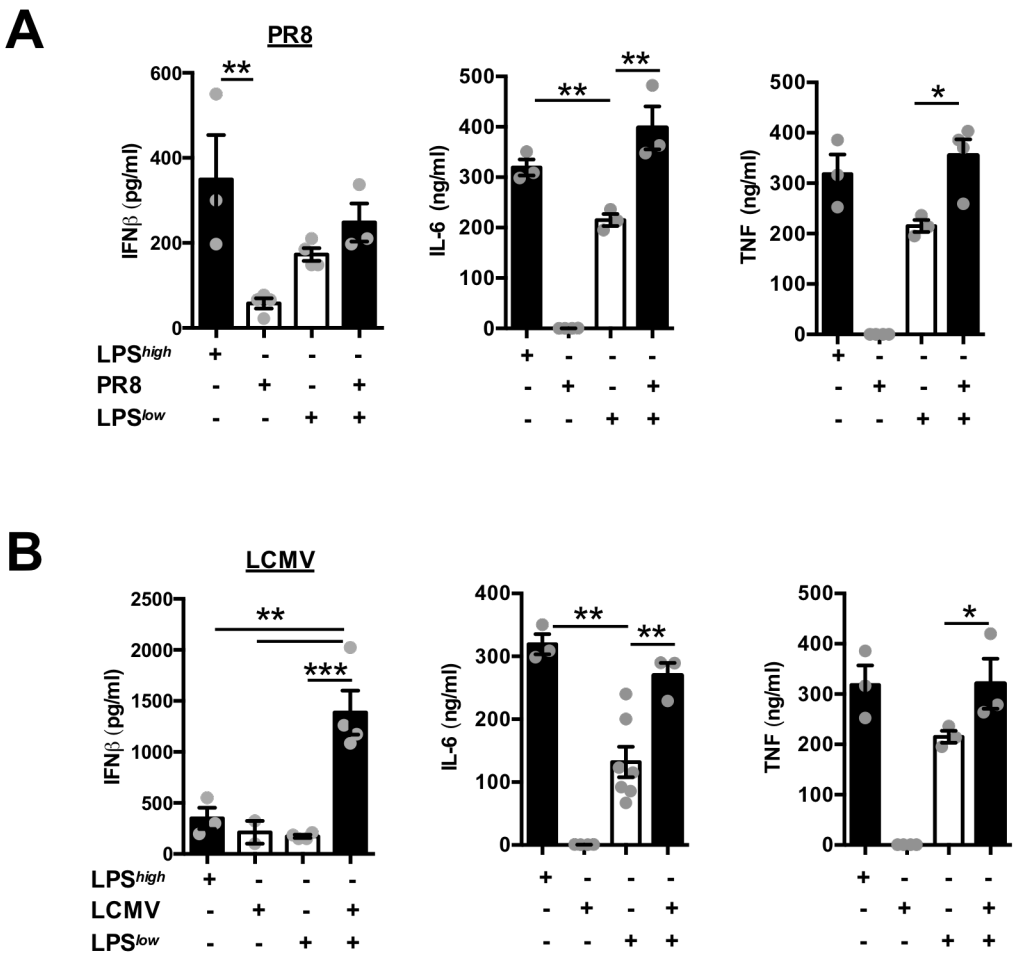


Supplementary Figure 1. Dose dependent LPS- and Poly (I:C) driven induction of preterm birth.

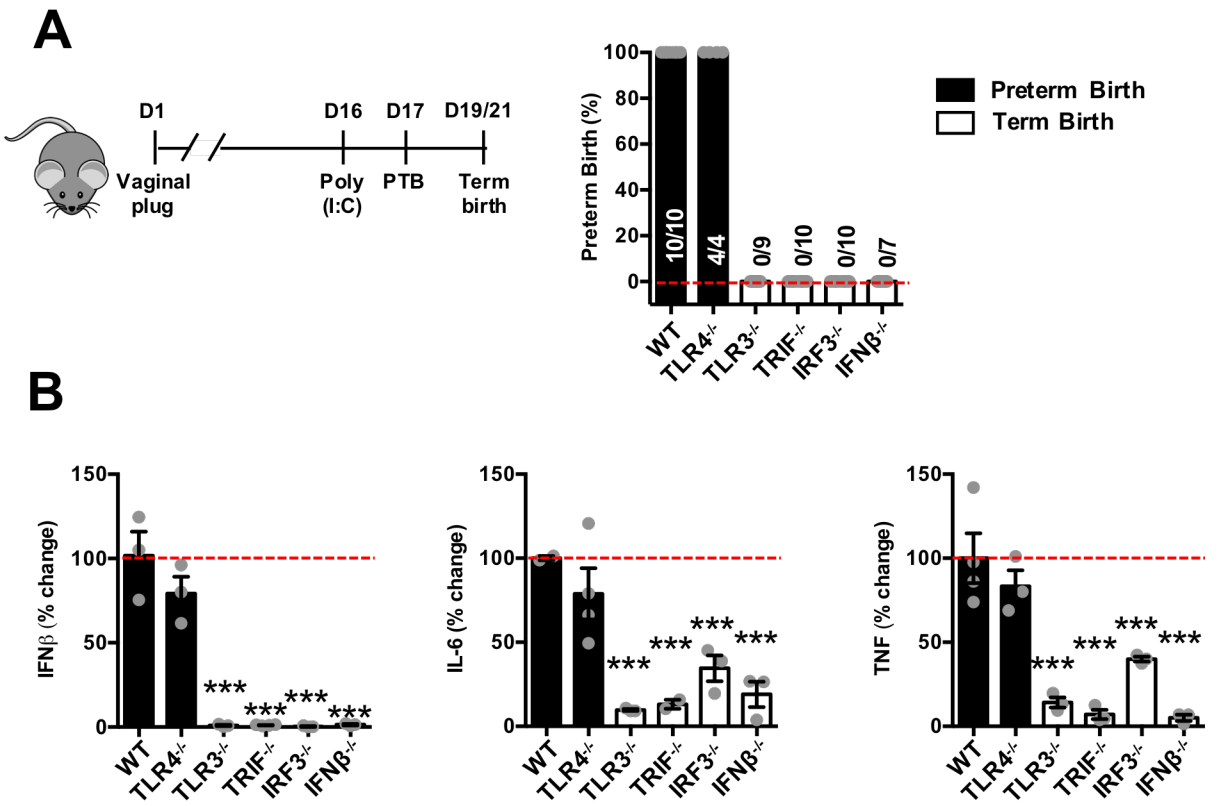
(A-B) Gravid WT mice were challenged with increasing concentrations of LPS and of Poly (I:C) on d16 of gestation, and the incidence of PTB was quantified. Data represent percent of induction of term or PTB.



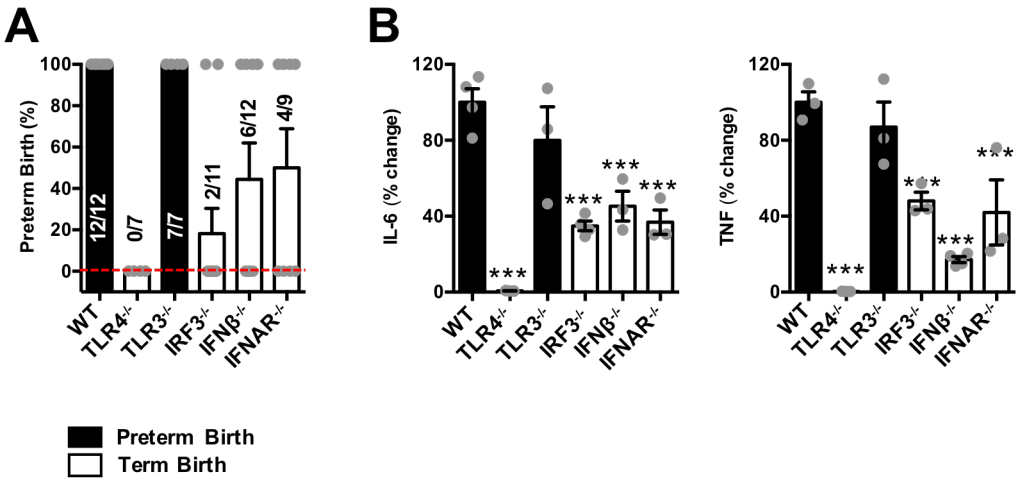
Supplementary Figure 2. Impact of subclinical pathogen infection priming for secondary inflammatory challenge-driven IFN-α production.
WT mice (n=3-6/condition) were mock-infected (saline) or infected with Influenza virus (PR8; $6*10^2$ PFU/mouse) or LCMV ($5*10^4$ PFU/mouse) for 24h or with *L. Monocytogenes* (Lm; WT, 1×10^2 CFU) for 24h prior to being mock-challenged or challenged with LPS^{low} for 4h and serum IFN-α levels were quantified by ELISA (PBL Interferon Source). ANOVA followed by Tukey's correction *P < 0.05, **P < 0.01, ***P < 0.001.



Supplementary Figure 3. Viral infection primes for secondary inflammatory challenge-driven cytokine production and induction of preterm birth in mice.
(A-B) WT mice (n=3-6/condition) were mock-infected or infected as described above (Figure 1A) with Influenza virus or LCMV for 48h prior to being mock-challenged or challenged with LPS^{low} for 4h and serum IFN-β, IL-6 and TNF levels were quantified by type I IFN activity assay and IVCCA respectively (actual values). ANOVA followed by Tukey's correction; *P < 0.05, **P < 0.01, ***P < 0.001. These data were used to calculate the % change of cytokine production over LPS^{low} (shown in Figure 1).

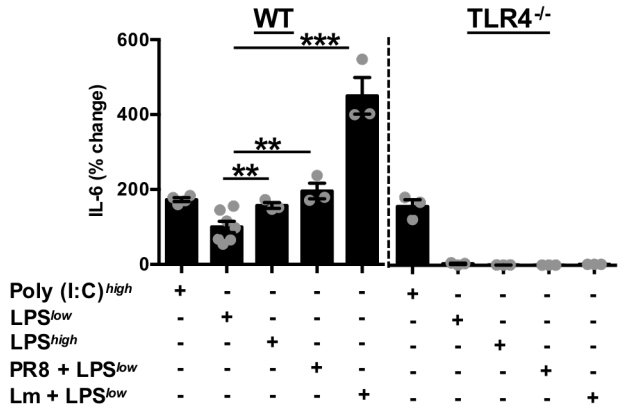


Supplementary Figure 4. Type I IFN/IFNAR axis is necessary for inflammatory challenge-driven cytokine production and induction of preterm birth in mice.
(A) A schematic overview of tractable preclinical model of PTB induction employed to define the ability of viral mimetic in driving PTB. Gravid mice were challenged Poly (I:C)^{high} on d16 of gestation, and the incidence of PTB was quantified. (B) WT mice (n=4-8/condition) were challenged with Poly (I:C) for 4h and IFN-β, IL-6 and TNF levels were quantified by type I IFN activity assay and IVCCA respectively. (A) Data represent percent of induction of term or PTB. (B) Dashed red line represents 100% induction of cytokine induction following Poly (I:C)^{low} alone challenge in WT mice. Data represent percent change over LPS^{low} (WT) + SE. (B) ANOVA followed by Tukey's correction; *P < 0.05, **P < 0.01, ***P < 0.001.

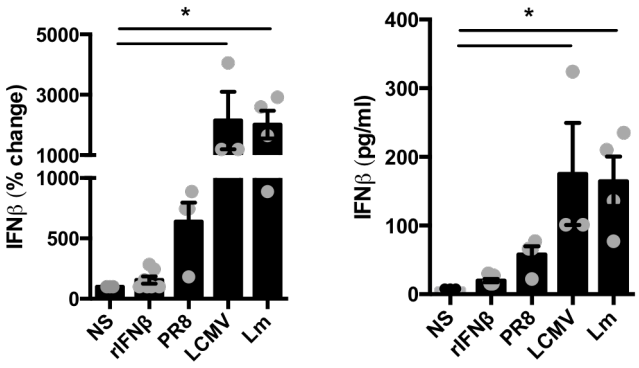


Supplementary Figure 5. Protection from LPS-driven PTB in mice lacking TLR4 and type I IFN signaling.

(A) Gravid WT and KO mice were challenged LPS^{high} on d16 of gestation, and the incidence of PTB was quantified. (B) WT and KO mice (n=3-4/condition) were challenged with LPS for 4h and IL-6 and TNF levels were quantified by IVCCA. Data represent percent change over LPS^{low} (WT) + SE. (B) ANOVA followed by Tukey's correction; *P < 0.05, **P < 0.01, ***P < 0.001.

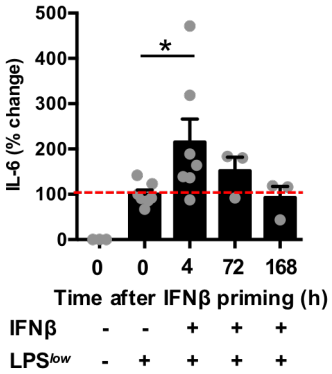


Supplementary Figure 6. Subclinical pathogen infection does not prime TLR4-deficient mice for secondary bacterial challenge proinflammatory cytokine production.
WT and TLR4$^{-/-}$ mice (n=3-6/condition) were with Influenza virus or Lm for 48h or 24h prior to being mock-challenged or challenged with LPS^{low} for 4h or Poly (I:C) alone (100 μg/mouse = low; 250 μg/mouse = high), or primed with Poly (I:C) for 4h prior to being challenged with LPS^{low} and serum IL-6 levels were quantified by IVCCA. Data represent percent change over LPS^{low} (WT) + SE. ANOVA followed by Tukey's correction *P < 0.05, **P < 0.01, ***P < 0.001.



Supplementary Figure 7. Physiological relevance of exogenous IFN-β levels utilized in our studies in comparison to subclinical pathogen infection.

WT mice were challenged with recombinant mouse IFN-β (r IFN-β; 10^4 U/mouse) for 8 hr or infected with Influenza virus (PR8; $6*10^2$ PFU/mouse) or LCMV ($5*10^4$ PFU/mouse) for 48h or with *L. Monocytogenes* (Lm; WT, 1×10^2 CFU) for 24h and serum IFN-β levels were quantified by ELISA. Data represent both as actual values and percent change over NS + SE as before. ANOVA followed by Tukey's correction *P < 0.05, **P < 0.01, ***P < 0.001.



Supplementary Figure 8. Transient IFN-β priming effects on IL-6 production in vivo.
WT mice (n=4-8/condition) were challenged with recombinant mouse IFN-β (10^4 U/mouse) for the indicated time points, LPS^{low} or were primed with IFN-β and challenged with LPS^{low} and serum IL-6 levels were quantified by IVCCA. Dashed red line represents 100% induction of cytokine induction following LPS^{low} alone challenge in WT mice. Data represent percent change over LPS^{low} (WT) + SE. ANOVA followed by Tukey's correction *P < 0.05, **P < 0.01, ***P < 0.001.