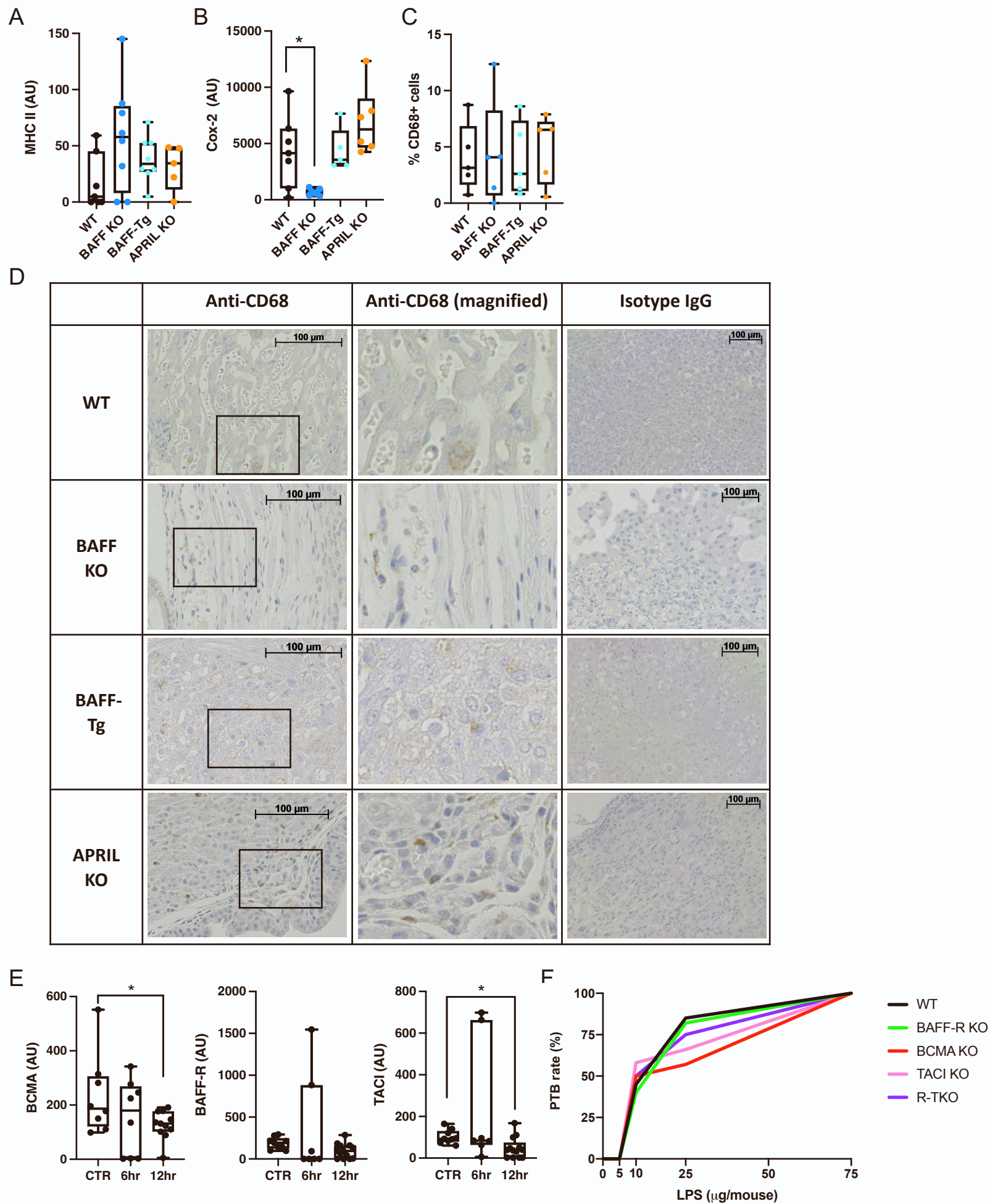


Supplemental information

**BAFF and APRIL counterregulate susceptibility
to inflammation-induced preterm birth**

Jessica R. Doll, Maria E. Moreno-Fernandez, Traci E. Stankiewicz, Jennifer L. Wayland, Adrienne Wilburn, Benjamin Weinhaus, Claire A. Chougnnet, Daniela Giordano, Monica Cappelletti, Pietro Presicce, Suhas G. Kallapur, Nathan Salomonis, Tamara Tilburgs, and Senad Divanovic



Supplementary Figure 1. BAFF contributes to induction of parturition, Related to Figure 2.

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(A) Decidua/myometrium *Ciita* (MHC II) mRNA expression in WT, BAFF deficient, BAFF-Tg, and APRIL deficient mice 6 hours following 75 µg LPS challenge (n = 4-8/condition).

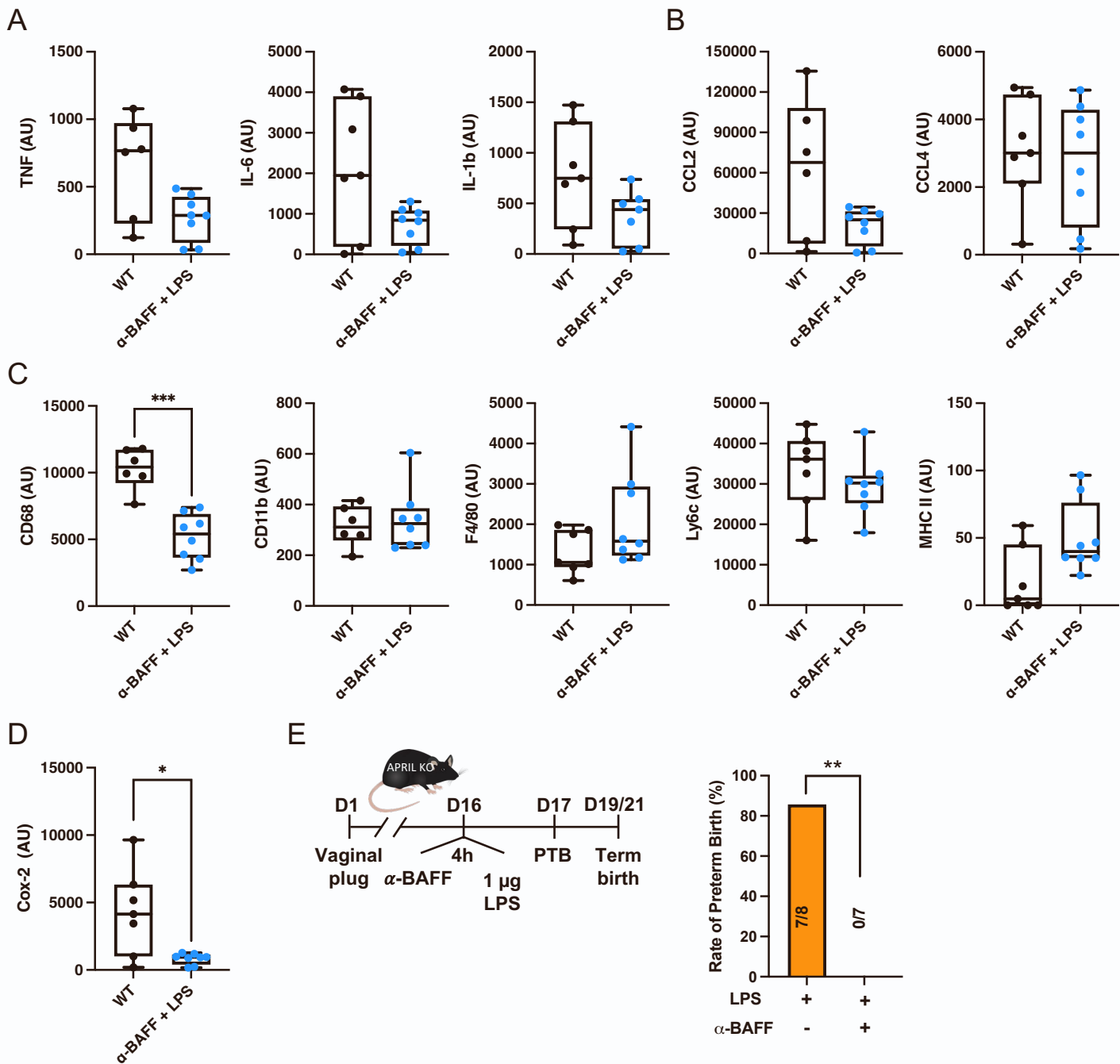
(B) Decidua/myometrium *Ptgs2* (Cox-2) mRNA expression in WT, BAFF deficient, BAFF-Tg, and APRIL deficient mice 6 hours following 75 µg LPS challenge. Student's t-test *p<0.05.

(C) Quantification of CD68 positive cells out of total nuclei from immunohistochemical detection in WT, BAFF deficient, BAFF-Tg, and APRIL deficient mouse myometrium/decidua/placenta 6 hours following saline (non-stimulated) injection (n = 3-4/condition).

(D) Immunohistochemical detection of CD68 (left and middle columns) or isotype control (right column) in WT, BAFF deficient, BAFF-Tg, and APRIL deficient mouse myometrium/decidua/placenta 6 hours following 75 µg LPS challenge (n = 3/condition). Left: Representative image, 20X objective. Middle: Indicated region of left image magnified to show detail (boxed region). Right: Representative isotype control image, 10X objective.

(E) Decidua/myometrium *Tnfrsf13c* (BAFF-R), *Tnfrsf17* (BCMA), and *Tnfrsf13b* (TACI) mRNA expression in WT mice 6 hours following 75 µg LPS challenge or saline injection (CTR) (n = 7-13/condition). Student's t-test *p<0.05.

(F) Gravid WT, BAFF-R deficient, BCMA deficient, TACI deficient, and BAFF-R/BCMA/TACI deficient (R-TKO) mice were injected intraperitoneally with LPS on day of 16 gestation and incidence of PTB was quantified (n = 4-24/condition). Chi-square (2x5 matrix): p=0.6820.



Supplementary Figure 2. Anti-BAFF treatment reduces induction of inflammatory signaling in the uterus, Related to Figure 3.

Supplementary Figure 2. Anti-BAFF treatment reduces induction of inflammatory signaling in the uterus, Related to Figure 3.

(A) Decidua/myometrium *Tnf*, *Il6*, and *Il1b* mRNA expression in WT mice treated with anti-BAFF (2 μ g/g) for 4 hours prior to 75 μ g LPS challenge for 6 hours. Data compared to WT mice treated with LPS alone (n = 6-8/condition).

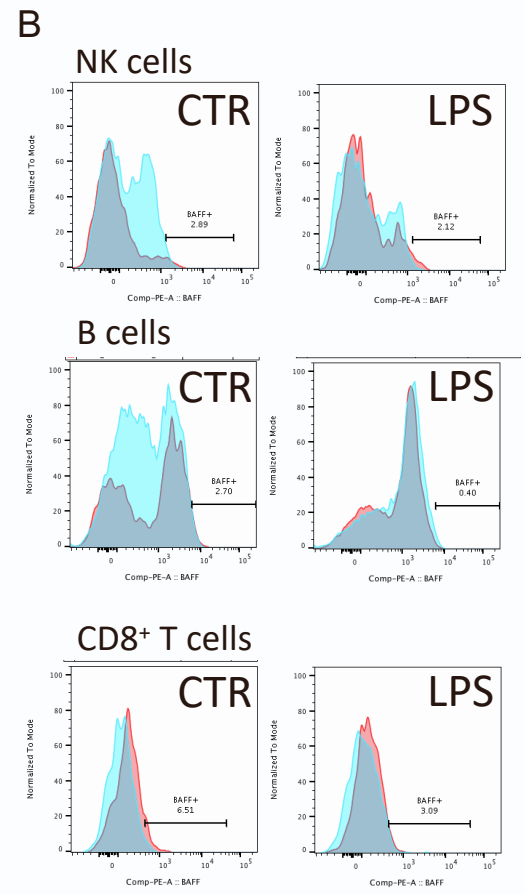
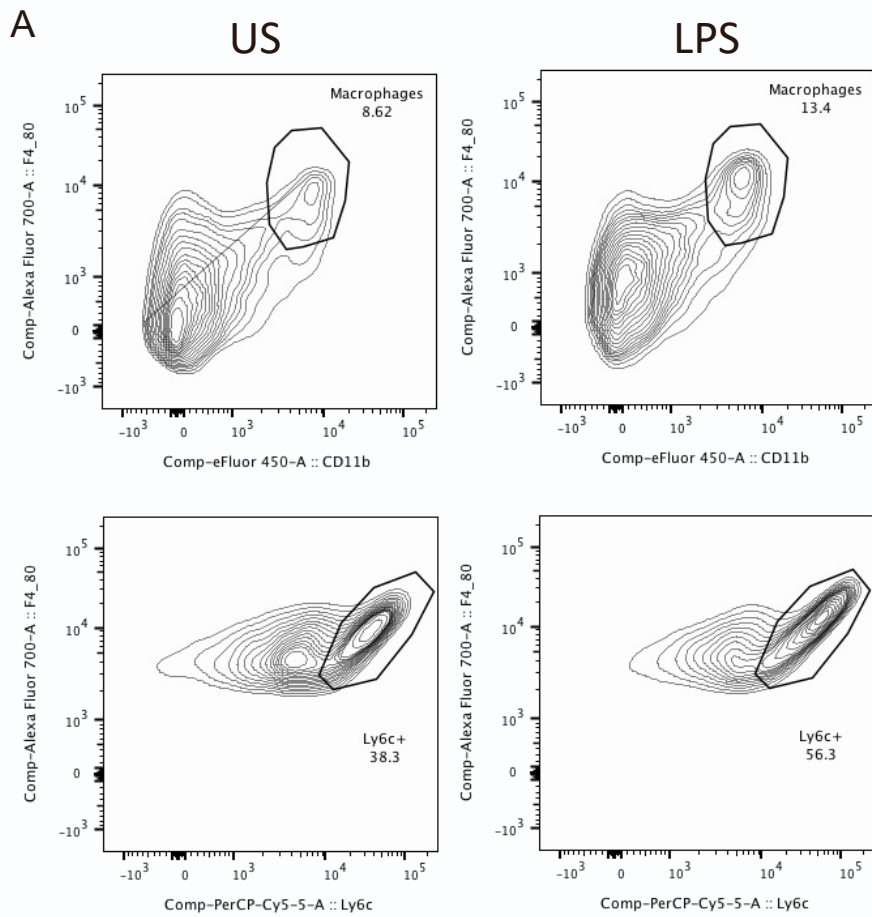
(B) Decidua/myometrium *Ccl2* and *Ccl4* mRNA expression in WT mice treated with anti-BAFF (2 μ g/g) for 4 hours prior to 25 μ g LPS challenge for 6 hours. Data compared to WT mice treated with LPS alone (n = 6-8/condition).

(C) Decidua/myometrium *Cd68*, *Cd11b*, *Adgre1* (F4/80), *Ly6c*, and *Ciita* (MHC II) mRNA expression in WT mice treated with anti-BAFF (2 μ g/g) for 4 hours prior to 25 μ g LPS challenge for 6 hours. Data compared to WT mice treated with LPS alone (n = 6-8/condition).

(D) Decidua/myometrium *Ptgs2* (Cox-2) mRNA expression in WT mice treated with anti-BAFF (2 μ g/g) for 4 hours prior to 25 μ g LPS challenge for 6 hours. Data compared to WT mice treated with LPS alone (n = 6-8/condition).

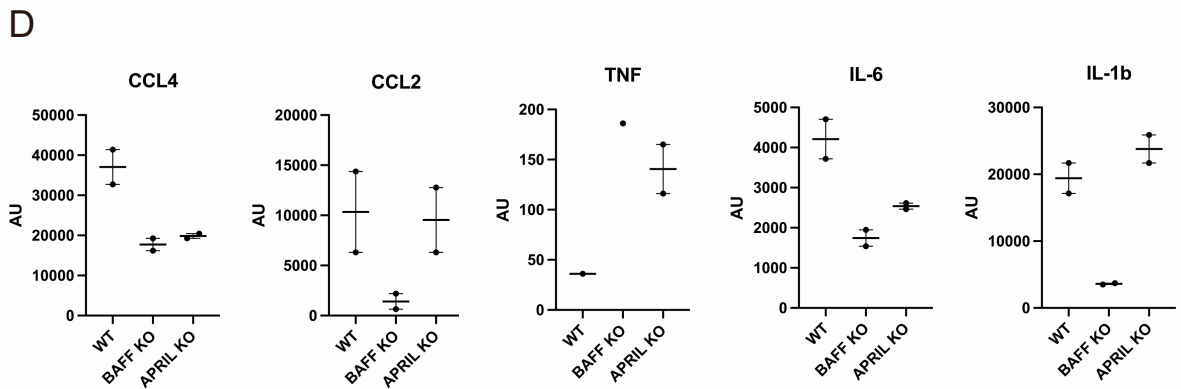
(E) Gravid APRIL deficient mice were injected intraperitoneally, on day 16 of gestation, with anti-BAFF (2 μ g/g) and 4 hours later were challenged with 1 μ g LPS. Incidence of PTB was quantified. Fisher's exact test p=0.0014.

(A-D) Student's t-test *p<0.05, **p<0.01.



C

Sample	RIN
WT LPS1	7.30
WT LPS2	6.60
BAFF KO LPS1	7.30
BAFF KO LPS2	7.10
APRIL KO LPS1	7.60
APRIL KO LPS2	7.60



Supplementary Figure 3. Macrophages are the primary BAFF-producing immune cell type, Related to Figure 4.

**Supplementary Figure 3. Macrophages are the primary BAFF-producing immune cell type,
Related to Figure 4.**

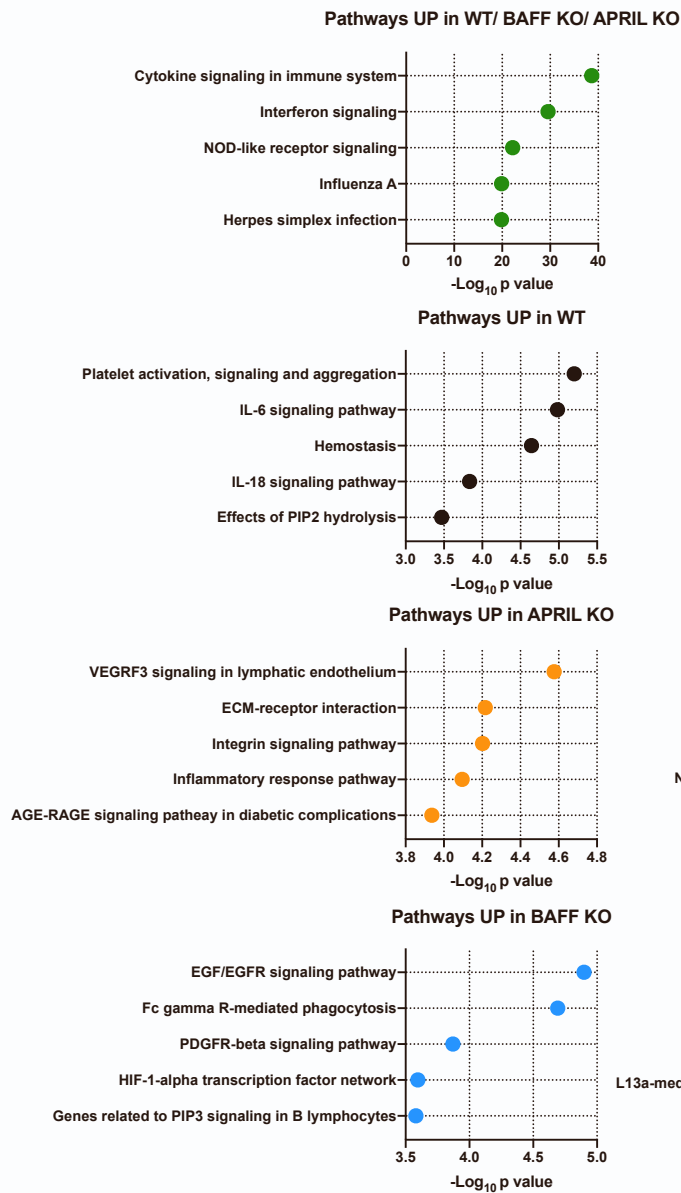
(A) Example of the gating strategy for decidua/myometrium macrophage (CD45⁺F4/80⁺CD11b⁺) populations and the activation marker Ly6c. Uterine tissue composed of decidua and myometrium was collected 6 hours post LPS or saline challenge (US = unstimulated) and analyzed by flow cytometry (n = 2/condition).

(B) BAFF expression levels in NK cells, B cells, and CD8⁺ T cells measured in BAFF reporter mice (red) over WT background (blue).

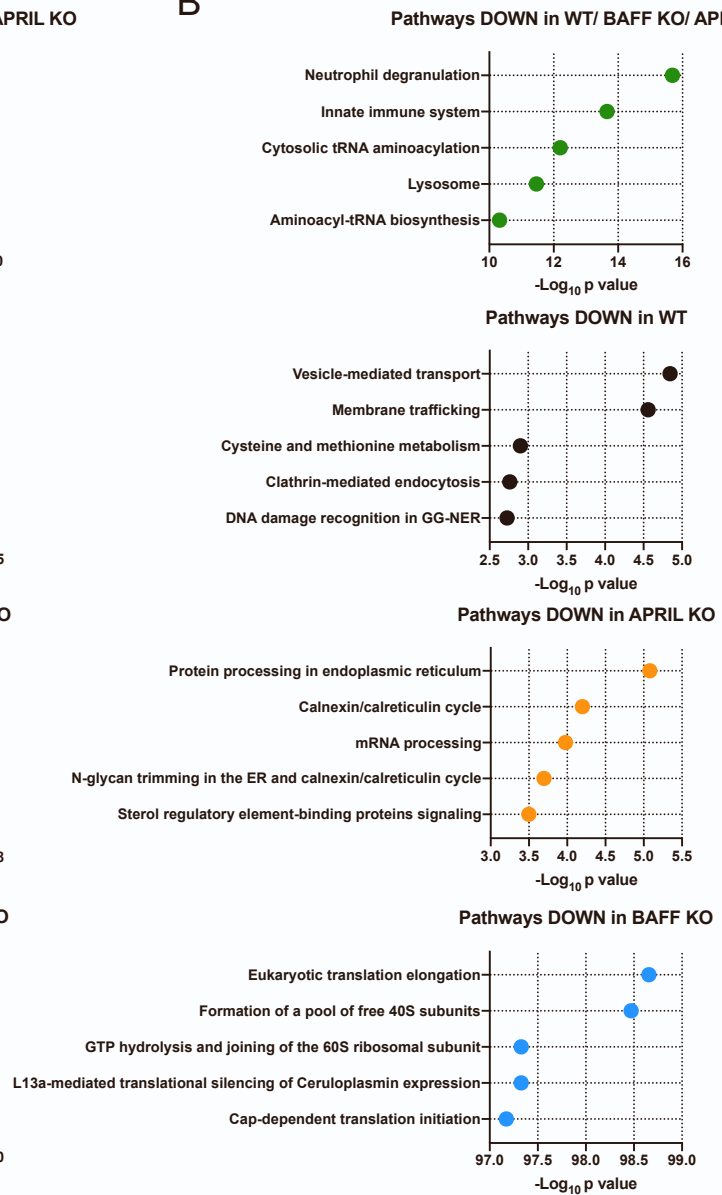
(C) RNA integrity numbers for samples used in RNA Seq (see **Figure 4D-J**).

(D) RT-qPCR values for selected genes.

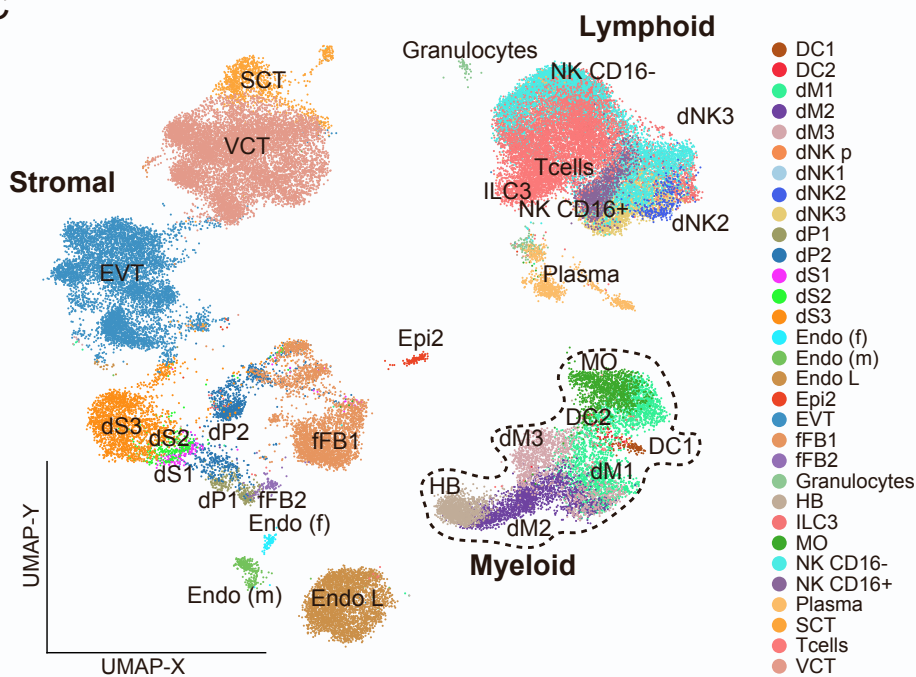
A



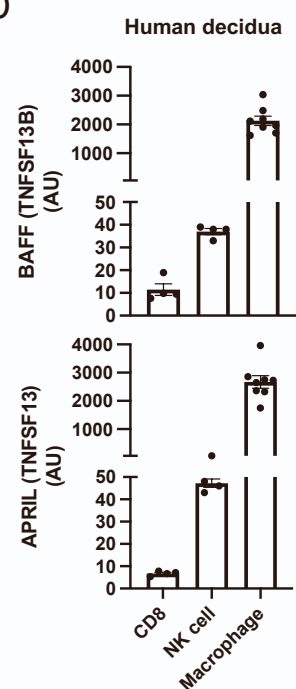
B



C



D



Supplementary Figure 4. Pathways up or downregulated following LPS stimulation of WT, APRIL KO, and BAFF KO macrophages, Related to Figure 4.

Supplementary Figure 4. Pathways up or downregulated following LPS stimulation of WT, APRIL KO, and BAFF KO macrophages, Related to Figure 4.

(A) Genes that were >1.5-fold upregulated were entered into ToppGene to identify potentially affected pathways.

(B) Genes that were >1.5-fold downregulated were entered into ToppGene to identify potentially affected pathways.

(C) UMAP of an integrated single-cell RNA-Seq dataset of term and preterm placenta from Pique-Regi et al. 2019, with cell-populations assigned from an independent placental cell atlas (Single-Cell-Portal- SCP860).

(D) BAFF and APRIL mRNA expression in first trimester human decidual tissue (n = 4-8/condition).

Oligonucleotides		
<i>Tnfrsf13b (Baff)</i>	BIO RAD	qMmuCID0005110
<i>Tnfrsf13 (April)</i>	BIO RAD	qMmuCED0003844
<i>Tnf</i>	BIO RAD	qMmuCED0004141
<i>β-actin</i>	BIO RAD	qMmuCED0027505
<i>Il6</i>	BIO RAD	qMmuCID0005613
<i>Il1b</i>	BIO RAD	qMmuCID0005641
<i>Cd68</i>	BIO RAD	qMmuCED0003822
<i>Ccl2</i>	BIO RAD	qMmuCED0048300
<i>Ccl4</i>	BIO RAD	qMmuCED0003869
<i>Ly6c</i>	BIO RAD	qMmuCID0061787
<i>Itgam (Cd11b)</i>	BIO RAD	qMmuCID0005971
<i>Clita (MHC II)</i>	BIO RAD	qMmuCID0040081
<i>Adgre1 (F4/80)</i>	BIO RAD	qMmuCID0017591
<i>Ptgs2 (Cox-2)</i>	BIO RAD	qMmuCED0003742
<i>Tnfrsf13c (BAFF-R)</i>	BIO RAD	qMmuCED0040620
<i>Tnfrsf17 (BCMA)</i>	BIO RAD	qMmuCID0023501
<i>Tnfrsf13b (TACI)</i>	BIO RAD	qMmuCED0045220

Table S4. Oligonucleotides used for gene expression analysis by RT-qPCR, Related to STAR Methods.