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**Supplemental Information**

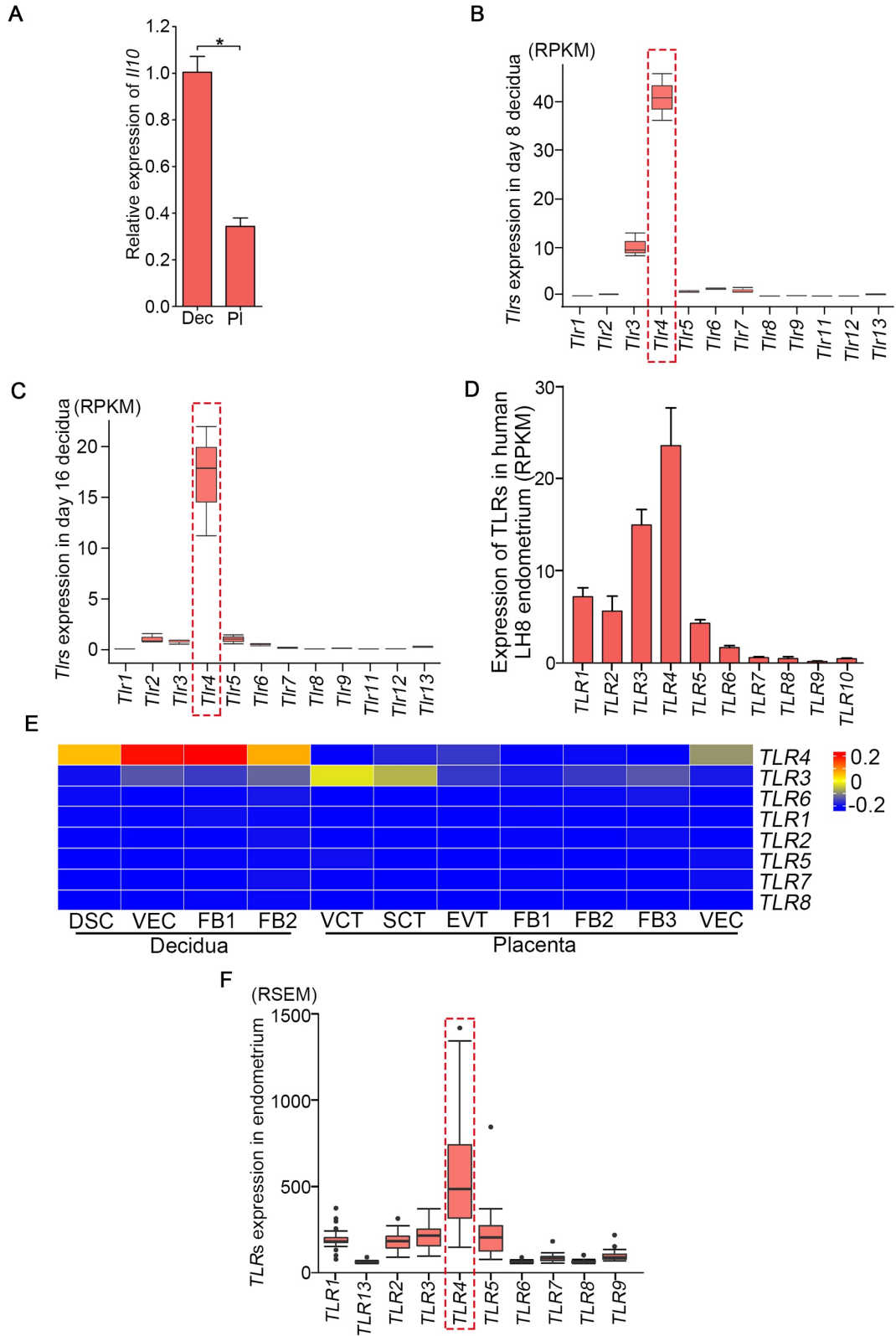
**Endothelial Cells in the Decidual Bed**

**Are Potential Therapeutic Targets**

**for Preterm Birth Prevention**

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# 1 Supplemental Information



3 **Figure S1, related to Figure 1. TLR4 is the dominant member expressed in**  
4 **the decidua during pregnancy**

5 (A) Relative expression of *Il10* in day 16 decidua (Dec) and placenta (Pl). n=3,  
6 \*p<0.05. Data are represented as mean ± SEM.

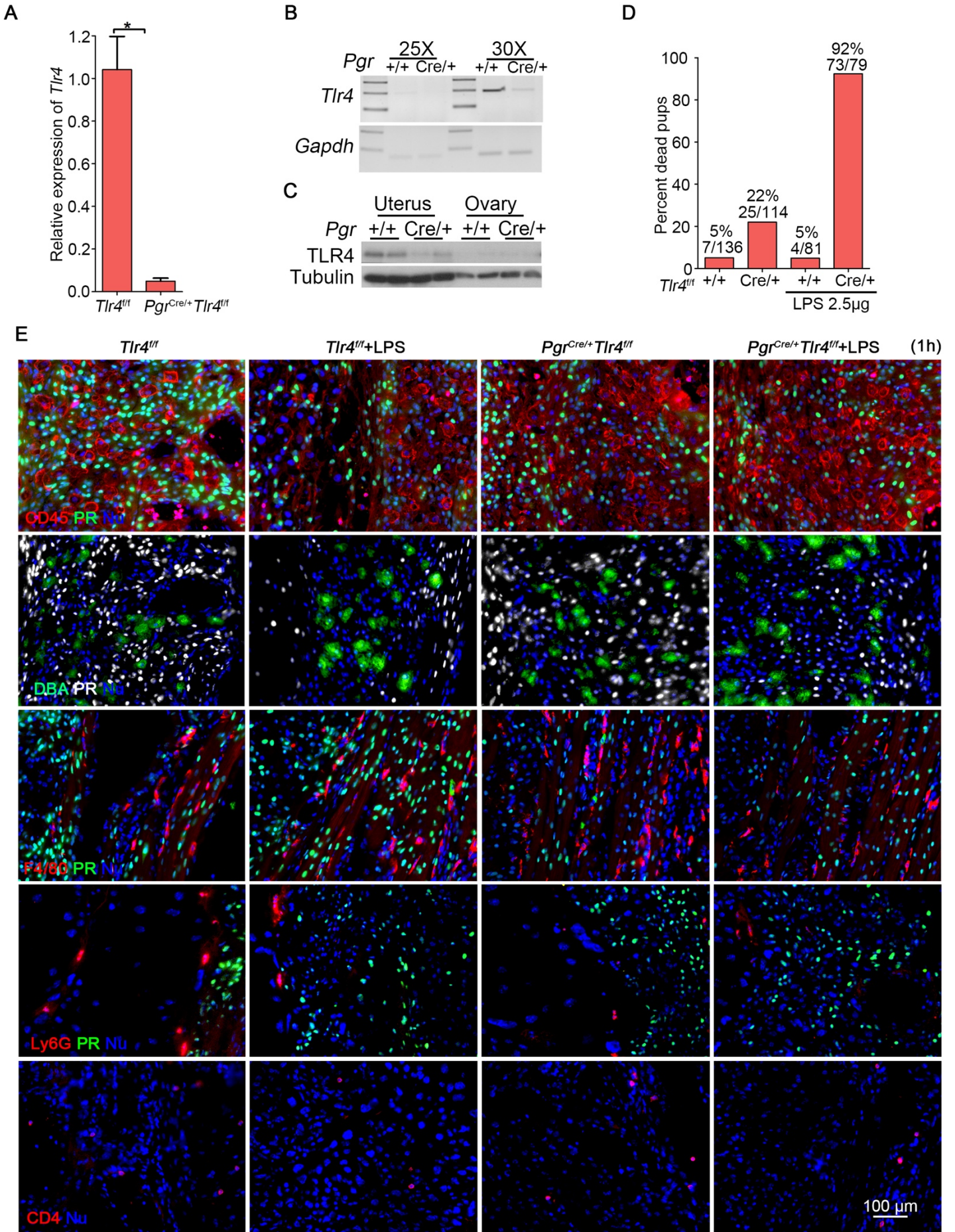
7 (B and C) Relative expression of the members of *Tlr* family in isolated stromal  
8 cells from days 8 and 16 and recalculated from published database. RPKM:  
9 reads per kilobase per million.

10 (D) Expression of *TLR* family in LH8 human endometrium. RPKM: reads per  
11 kilobase per million.

12 (E) Expression of *TLR* family in different cell types from single-cell sequencing in  
13 first trimester decidua and placenta. DSC: decidual stromal cells; VEC:  
14 vascular endothelial cells; FB: fibroblast cells; VCT: villous cytotrophoblasts;  
15 SCT: syncytiotrophoblasts; EVT: extravillous trophoblasts

16 (F) Expression of all *TLR* members in normal human endometrium from human  
17 TCGA database. RSEM: RNA-Seq by Expectation-Maximization.

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20 **Figure S2, related to Figure 2. TLR4 deletion in uterus by Pgr-Cre**

21 (A) Real-Time qPCR of *Tlr4* expression in *Tlr4<sup>fl/fl</sup>* and *Pgr<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>* mice in uterus.

22 (B) RT-PCR of *Tlr4* expression in *Tlr4<sup>fl/fl</sup>* and *Pgr<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>* mice at 25 and 30 cycles.

23 *Gapdh* was used as an internal control.

24 (C) Western blotting of TLR4 in the uterus and ovary from *Tlr4<sup>fl/fl</sup>* and *Pgr<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>*

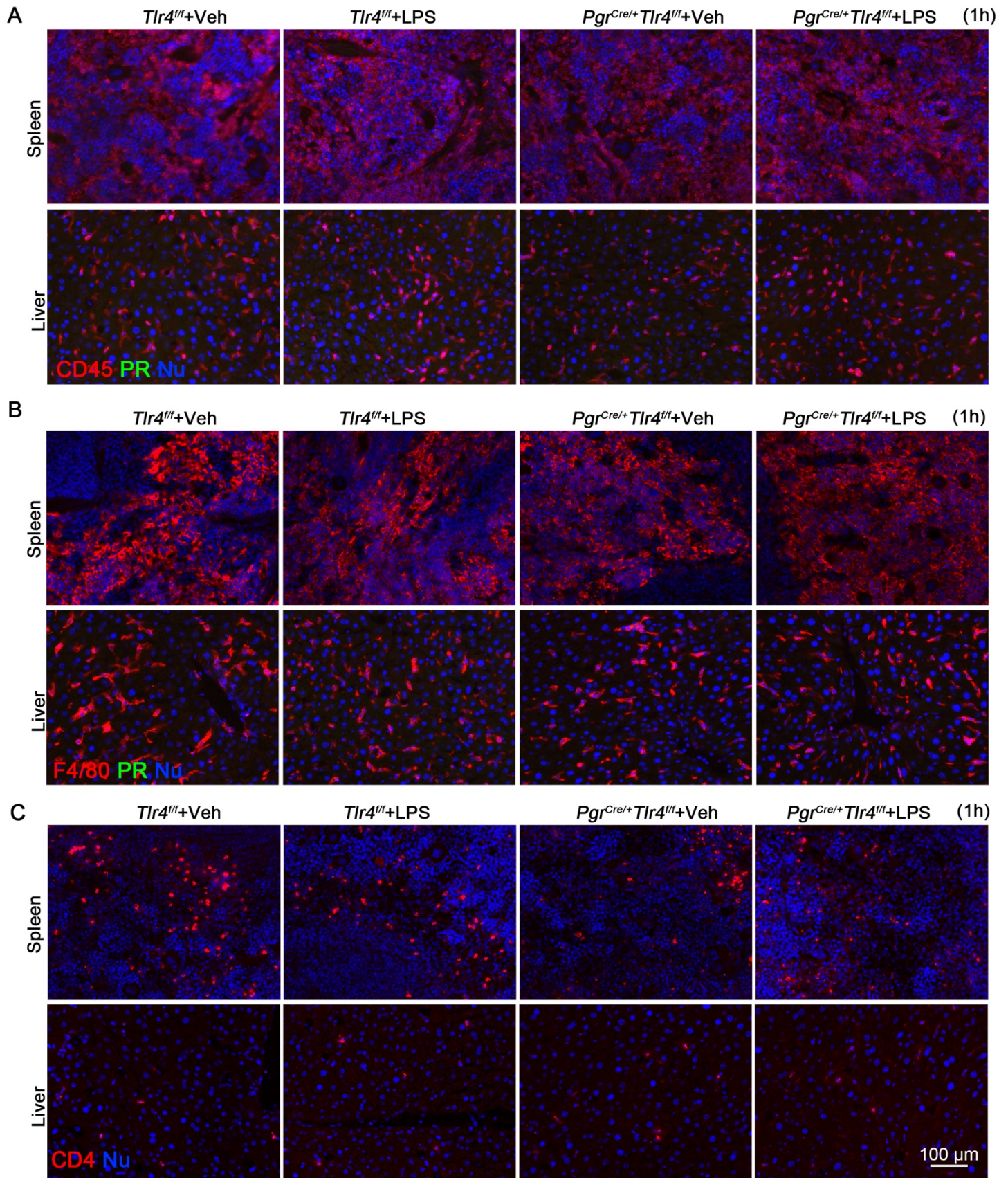
25 mice on day 8 of pregnancy.

26 (D) The ratio of dead pups to total number of pups/gestations in *Tlr4<sup>fl/fl</sup>* and

27 *Pgr<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>* mice in the presence or absence of low dose LPS (2.5 µg/mouse).

28 (E) Staining of CD45, DBA-lectin (uNK cells), F4/80, Ly6G, CD4 and PR in day 16

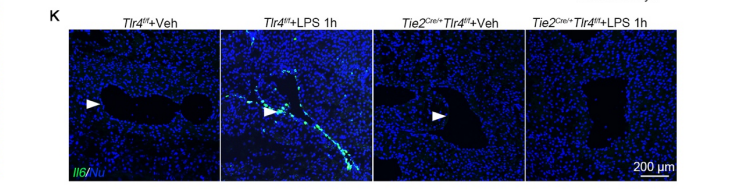
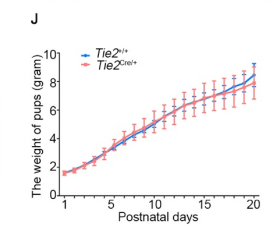
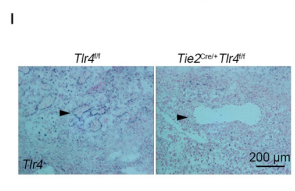
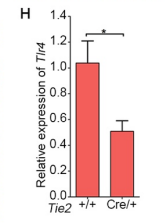
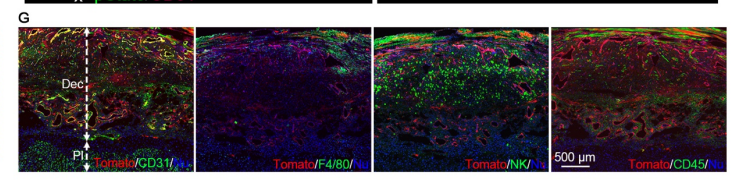
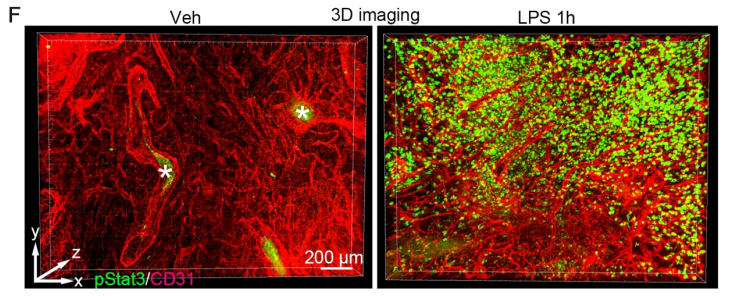
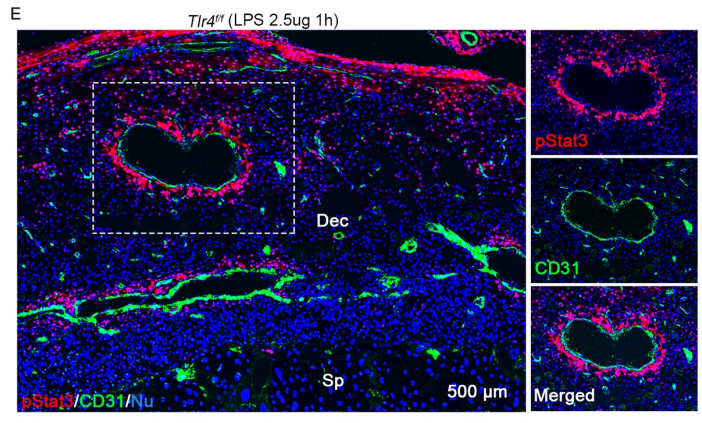
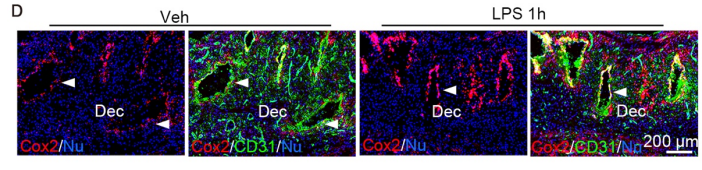
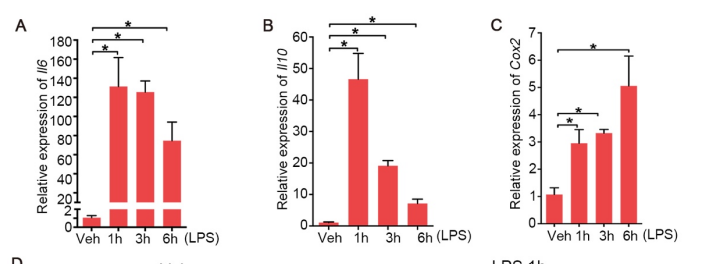
29 deciduae with or without LPS exposure for 1h. Scale bar, 100 µm



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33 **Figure S3, related to Figure 2. Expression of PR and CD45, F4/80 in the**  
34 **spleen and liver sections after LPS treatment in *Tlr4<sup>fl/fl</sup>* and *Pgr<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>***  
35 **deciduae**  
36 (A) Co-staining of CD45 and PR in *Tlr4<sup>fl/fl</sup>* and *Pgr<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>* spleen and liver with or  
37 without LPS exposure.  
38 (B) Co-staining of F4/80 and PR in *Tlr4<sup>fl/fl</sup>* and *Pgr<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>* spleen and liver with or  
39 without LPS exposure.  
40 (C) Co-staining of CD4 and PR in *Tlr4<sup>fl/fl</sup>* and *Pgr<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>* spleen and liver with or  
41 without LPS treatment.  
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48 **Figure S4, related to Figure 2 and 3. LPS induces cytokines in the decidua**  
49 (A-C) Relative expression of *Il6* (A), *Il10* (B) and *Cox2* (C), respectively, in *Tlr4<sup>ff</sup>*  
50 mice after treatment with LPS for 1, 3 and 6 hours, n = 4, \*, p<0.05. Data are  
51 represented as mean ± SEM.

52 (D) Co-immunostaining of *Cox2* (red) and CD31 (green) in *p53<sup>ff</sup>* deciduae after  
53 LPS treatment for 1 h. Dec: decidua. Arrowheads indicate endothelial cells.  
54 Scale bar, 200 µm.

55 (E) Co-immunofluorescence staining of pStat3 (red) and CD31 (green) in LPS-  
56 treated deciduae of *p53<sup>ff</sup>* mice. Images in the right panels depict the images  
57 in the demarcated rectangles. Scale bar, 500 µm. Dec, deciduae; Sp,  
58 spongiotrophoblast.

59 (F) 3D Co-immunofluorescence staining of pStat3 (red) and CD31 (green) in the  
60 LPS-treated decidua in *p53<sup>ff</sup>* mice. Scale bar, 100 µm.

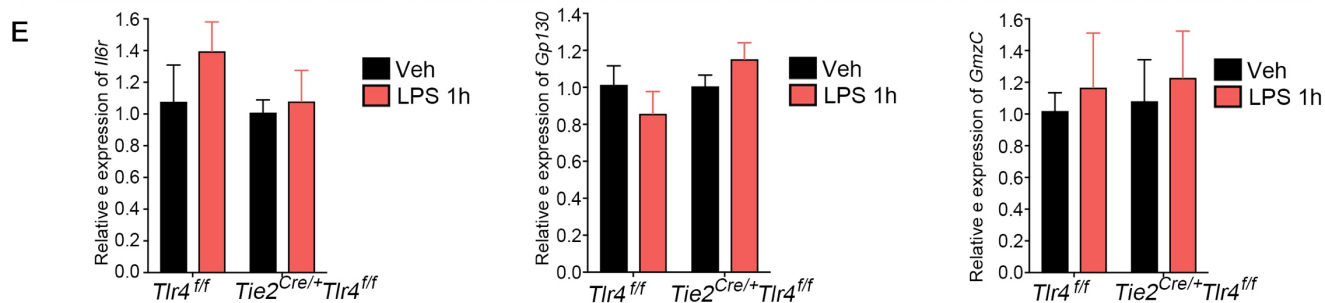
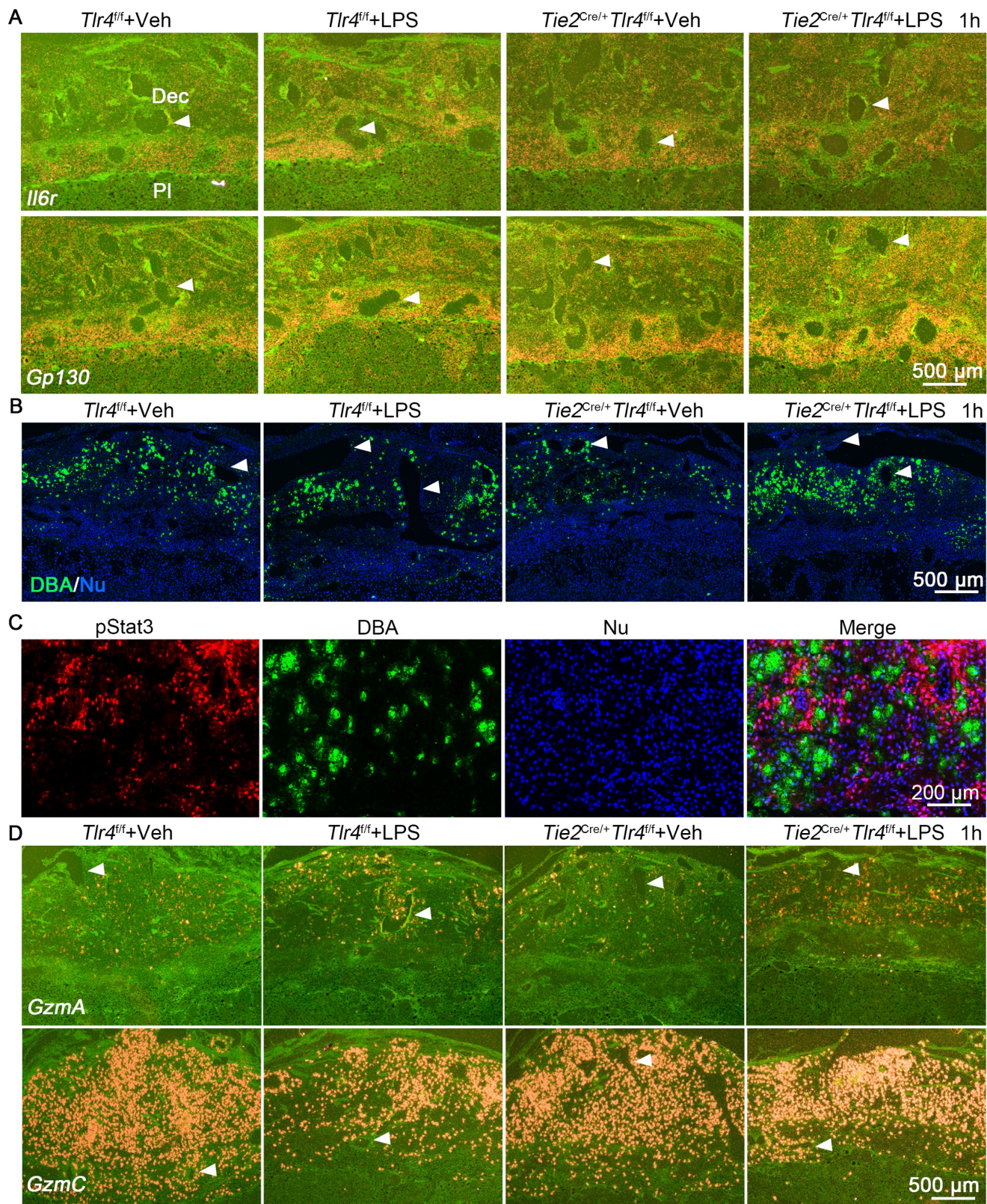
61 (G) Staining of immune cells and endothelial cells markers in *Tie2<sup>Cre/+</sup>Rosa<sup>Tomato</sup>*  
62 reporter mice. Dec: decidua, Pl: placenta, Scale bar, 500 µm.

63 (H) Relative expression of *Tlr4* in day 16 deciduae in *Tlr4<sup>ff</sup>* and *Tie2<sup>Cre/+</sup>Tlr4<sup>ff</sup>* mice.  
64 n=4, \*, p<0.05. Data are represented as mean ± SEM.

65 (I) DIG-In situ hybridization of *Tlr4* in *Tlr4<sup>ff</sup>* and *Tie2<sup>Cre/+</sup>Tlr4<sup>ff</sup>* mice. Arrow heads  
66 depict the location of endothelial cells. Scale bar, 200 µm.

67 (J) The weight of pups (gram) from days 1 to 20 after parturition between *Tlr4<sup>ff</sup>*  
68 and *Tie2<sup>Cre/+</sup>Tlr4<sup>ff</sup>* mice (n=5).

69 (K) LPS-induced expression of *Ilf6* by fluorescence *in situ* hybridization in the  
70 endothelial cells in day 16 deciduae in *Tlr4<sup>fl/fl</sup>* and *Tie2<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>* mice. Arrow  
71 heads indicate endothelial cells. Scale bar, 200  $\mu\text{m}$ .



73 **Figure S5, related to Figure 4. In situ hybridization of *Il6*, *Gp130* and**  
74 **granzymes and co-staining of pStat3 and DAB-lectin in day 16 *Tlr4<sup>fl/fl</sup>* and**  
75 ***Tie2<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>* deciduae.**

76 (A) In situ hybridization for *Il6r* and *Gp130* in *Tlr4<sup>fl/fl</sup>* and *Tie2<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>* decidua on  
77 day 16 after LPS (40 µg /mouse) treatment. Dec, decidua; PI, Placenta. Scale  
78 bar, 500 µm.

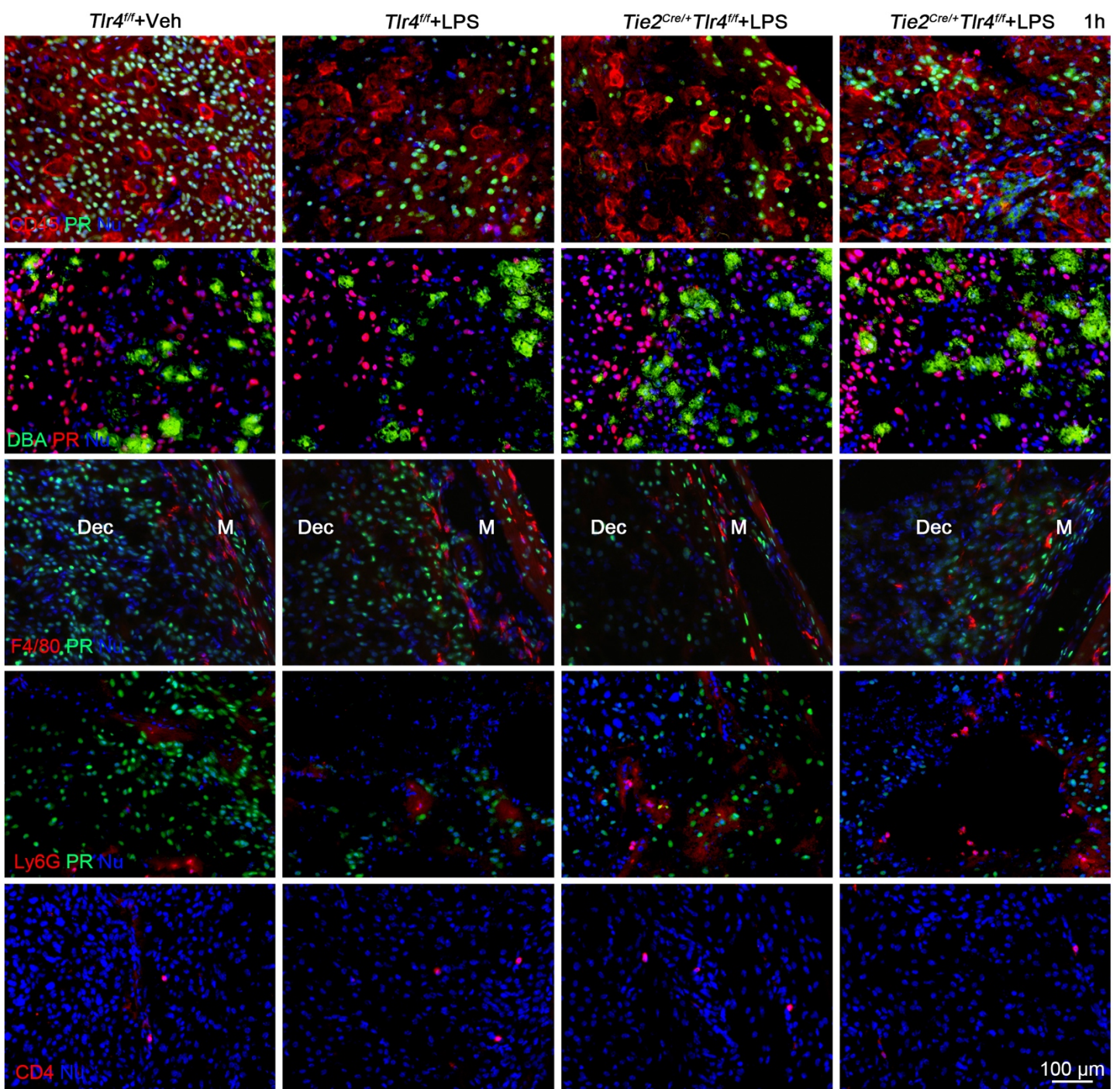
79 (B) Immunolocalization of DBA+ NK cells (green) in *Tlr4<sup>fl/fl</sup>* and *Tie2<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>*  
80 deciduae on day 16 in the absence or presence of LPS (40 µg /mouse)  
81 treatment. Scale bar, 500 µm.

82 (C) Co-immunolocalization of pStat3 (red) and DBA+ NK cells (green) in *Tlr4<sup>fl/fl</sup>*  
83 deciduae on day 16 after LPS (40 µg /mouse) treatment. Scale bar, 200 µm.

84 (D) In situ hybridization for *GzmA* and *GzmC* in *Tlr4<sup>fl/fl</sup>* and *Tie2<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>* deciduae  
85 on day 16 after LPS (40 µg /mouse) treatment. Scale bar, 500 µm.

86 (E) Quantification of *Il6r*, *Gp130* and *GzmC* in *Tlr4<sup>fl/fl</sup>* and *Tie2<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>* deciduae on  
87 day 16 after LPS (40 µg /mouse) treatment (n = 4). Relative expression is  
88 normalized against *rpL7* (a house-keeping gene).

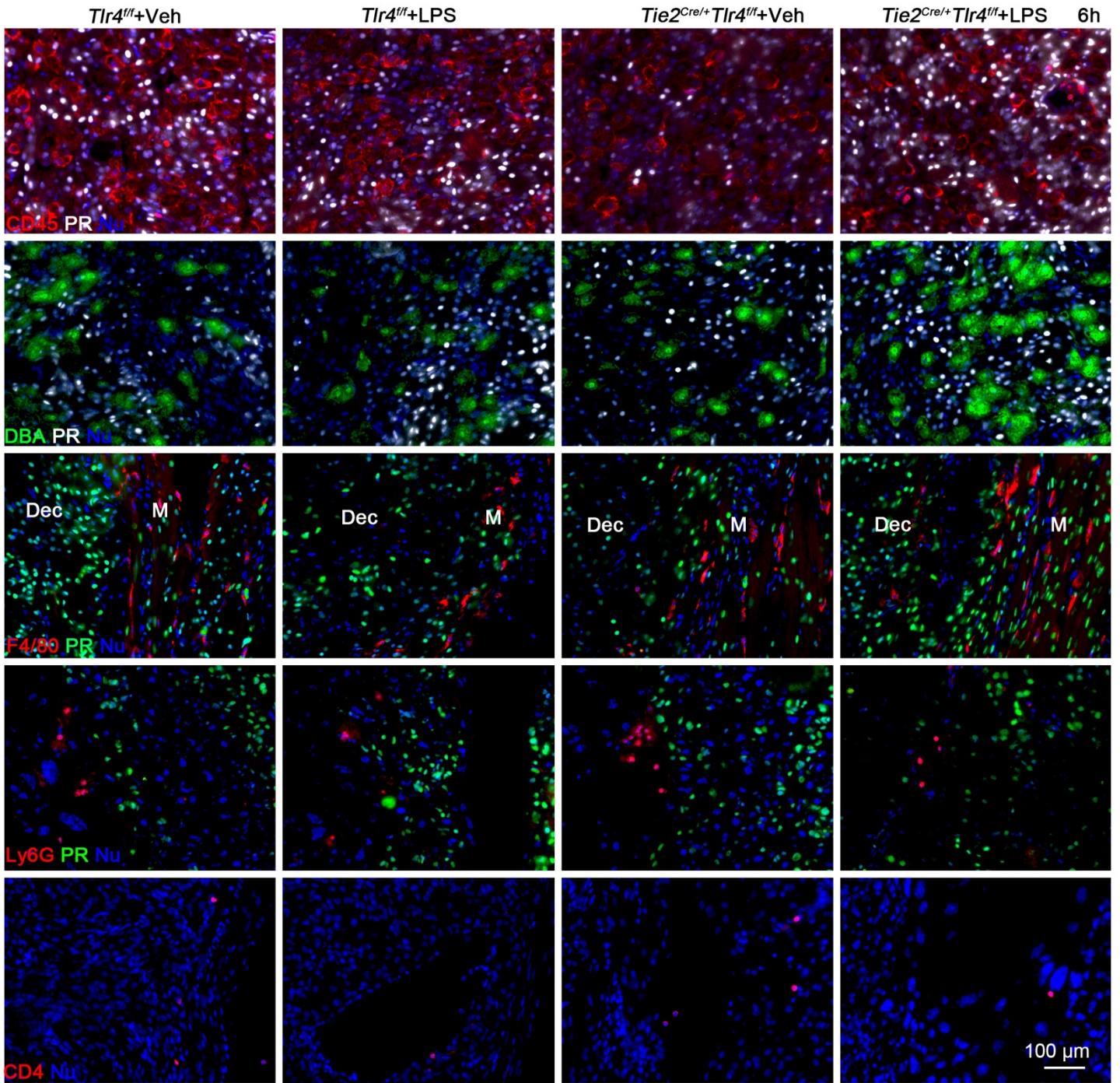
89 Arrow heads indicate blood vessels in all images.



94 **Figure S6, related to Figure 4. Distribution of immune cell identified by**  
95 **specific marker in *Tlr4<sup>fl/fl</sup>* and *Tie2<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>* deciduae after 1 hour of LPS**  
96 **treatment.**

97 Co-staining of CD45, DBA-lectin, CD4, Ly6G and F4/80 with PR in *Tlr4<sup>fl/fl</sup>* and  
98 *Tie2<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>* deciduae on day 16 after LPS (40 µg /mouse) treatment for 1 hour.

99 Dec, deciduae; M, myometrium. Scale bar for all images, 100 µm.



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**Figure S7, related to Figure 4. Distribution of immune cell identified by specific marker in *Tlr4<sup>fl/fl</sup>* and *Tie2<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>* deciduae after 6 hours of LPS treatment.**

Co-staining of CD45, DBA-lectin, CD4, Ly6G and F4/80 with PR in *Tlr4<sup>fl/fl</sup>* and *Tie2<sup>Cre/+</sup>Tlr4<sup>fl/fl</sup>* deciduae on day 16 after LPS (40 µg /mouse) treatment for 6 hours. Dec, deciduae; M, myometrium. Scale bar for all images, 100 µm.