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Supplemental information

Maternal-fetal conflict averted by progesterone-induced FOXP3+ regulatory T cells

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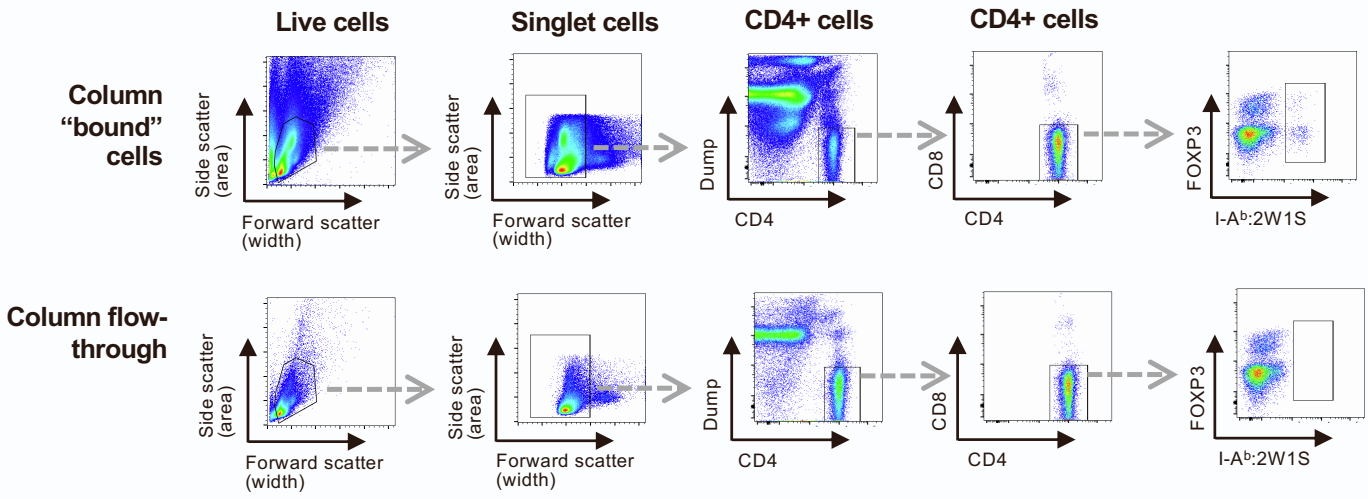

Figure S1. Flow cytometry analysis for identification of endogenous CD4+ cells with I-A^b:2W1S$_{55-68}$ specificity, Related to Figure 1. Single cell suspensions of spleen and lymph nodes were combined, stained with I-Ab:2W1S tetramer and anti-fluorochrome antibody beads, and enriched through magnetic columns. The column bound fraction and flow-through were separately stained using fluorochrome conjugated antibodies for cell surface and intracellular/intranuclear staining, and analyzed using the following gating approach. Dump panel includes anti-F4/80, anti-CD11b, anti-B220, anti-CD11c antibodies.

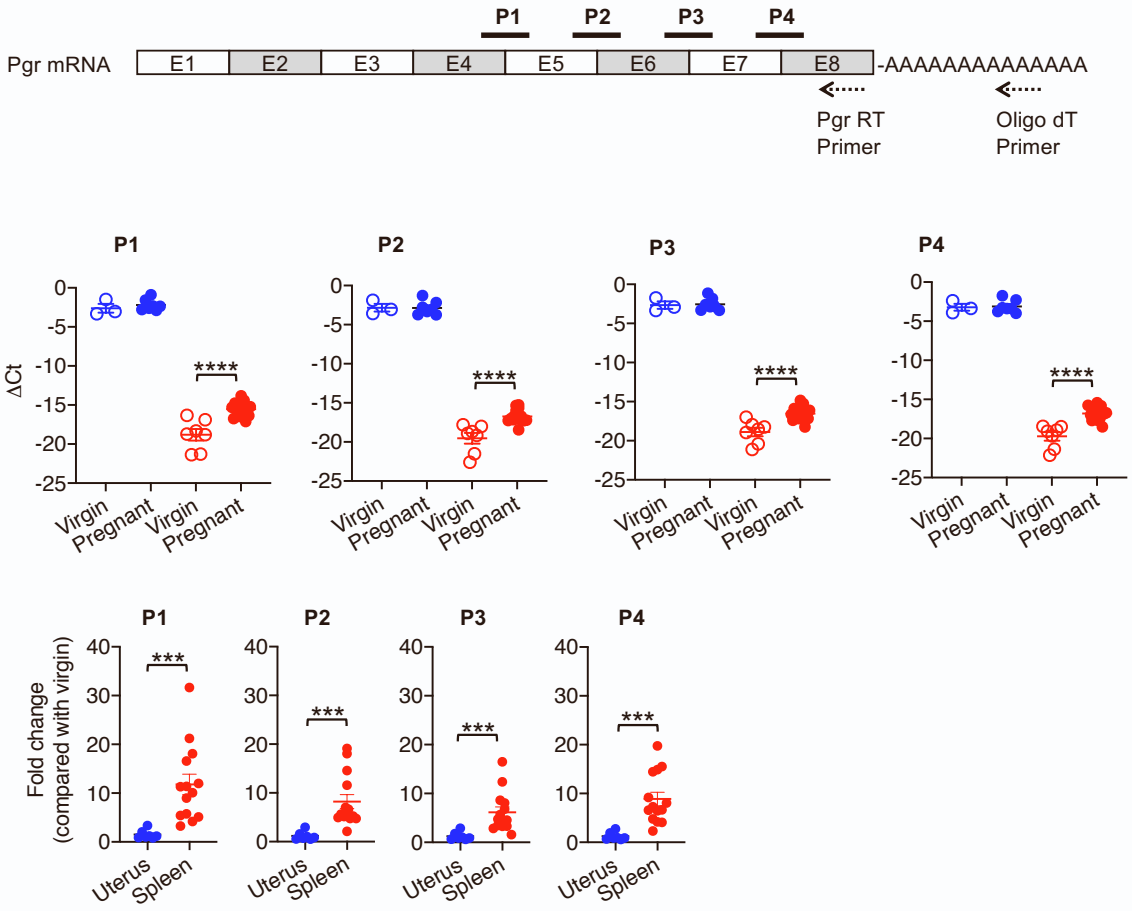

Figure S2. Pregnancy induced nuclear progesterone receptor expression amongst splenocytes, related to Figure 1. Schematic illustrating the 8 exons of nuclear progesterone receptor mRNA and exon-spanning probes (P1, P2, P3, P4) used for detection by RT-PCR (top). Nuclear progesterone receptor mRNA levels in the uterus (blue) and spleen (red) of virgin compared with midgestation (E11.5) pregnant mice. The ΔCT was calculated for each sample by subtracting the PR probe CT value from the Beta Actin CT value for the same sample. PR fold-change during pregnancy in the uterus and spleen was calculated using the $2^{-\Delta\Delta CT}$ method and normalizing to the average ΔCT value of virgin samples for each tissue. These data are from at least three independent experiments each with similar results, with each point representing data from an individual mouse. Bar, mean ± SEM. ***p<0.005, ****p<0.001

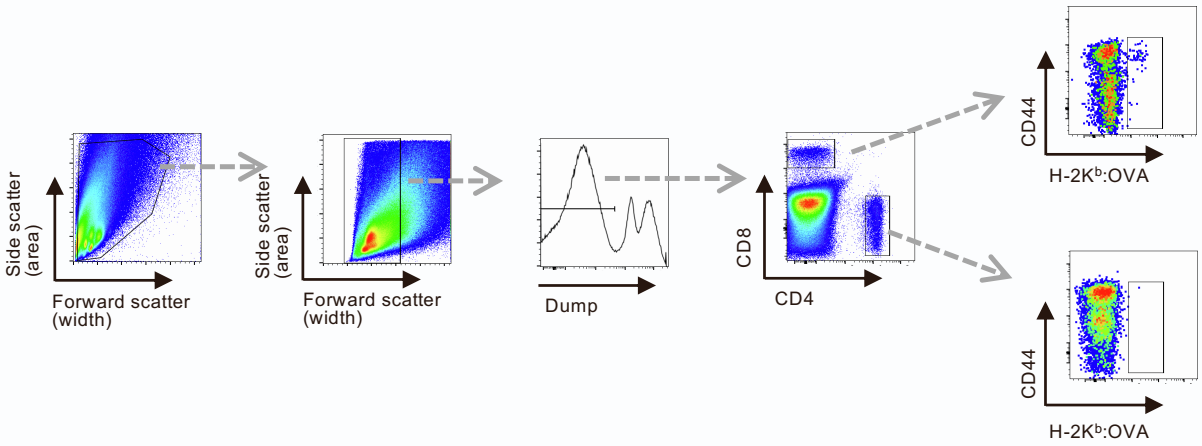

Figure S3. Flow cytometry analysis for identification of decidual CD8+ cells with H-2K^{b}:OVA$_{257-264}$ specificity, related to Figure 3. Single cell suspensions of the decidua of each concepti were combined from C57BL/6 mice during allogeneic pregnancy sired by 2W1S-OVA expressing males on the Balb/c background, stained with fluorochrome conjugated H-2Kb:OVA tetramer plus antibodies for cell surface and intracellular/intranuclear staining, and analyzed using the following gating approach, with CD4+ cells serving as a negative control. A similar approach staining with fluorochrome conjugated I-Ab:2W1S tetramer was used for identification of decidual CD4+ cells with I-A^{b}:2W1S specificity by gating on CD4+ cells, and using CD8+ cells as a negative control. Dump panel includes anti-F4/80, anti-CD11b, anti-B220, anti-CD11c antibodies.