

Expanded View Figures

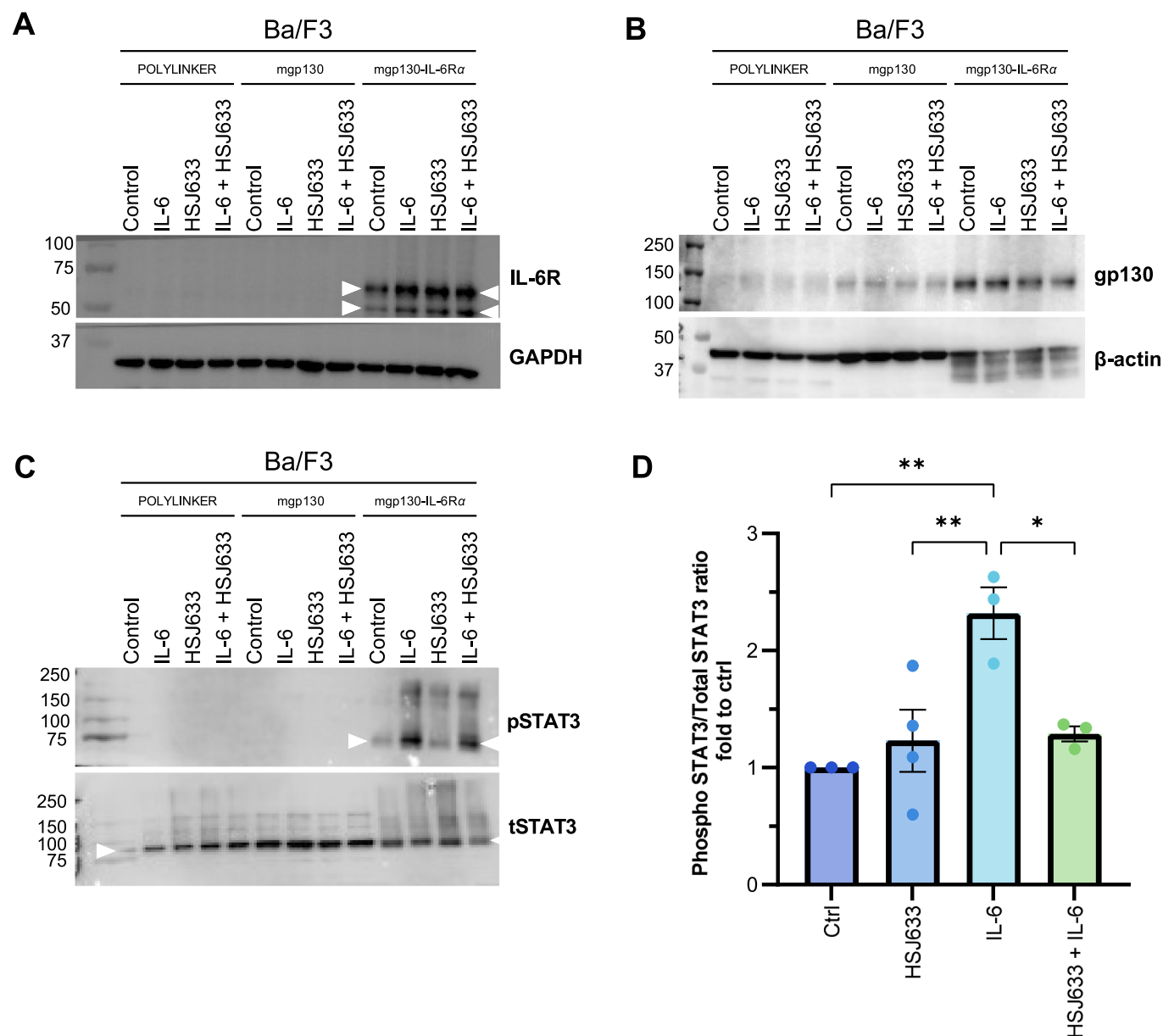


Figure EV1. HSJ633 reduces STAT3 activation in murine Ba/F3-mgp130-IL-6Rα cells but not in murine cells without IL-6Rα.

Representative Western blot displaying the levels of IL-6Rα, gp130 and STAT3 in Ba/F3-polylinker, Ba/F3-mgp130, and Ba/F3-mgp130-IL-6Rα cells treated with IL-6 (1.92 nM), HSJ633 (0.1 mM) and HSJ633 + IL-6. (A) Western blot image showing IL-6Rα and GAPDH bands. (B) Western blot image showing gp130 and β-actin bands. (C) Western blot image showing phospho-STAT3 and total STAT3 bands. (D) Quantification of STAT3 activation in Ba/F3-mgp130-IL-6Rα, presented as mean ± SEM of 3-4 samples per group. Densitometric analysis of the bands indicates a significant reduction in STAT3 activation in HSJ633 + IL-6-treated cells relative to IL-6. One-way ANOVA with Dunnett's multiple comparisons test compared to IL-6. * $P < 0.05$, ** $P < 0.01$.

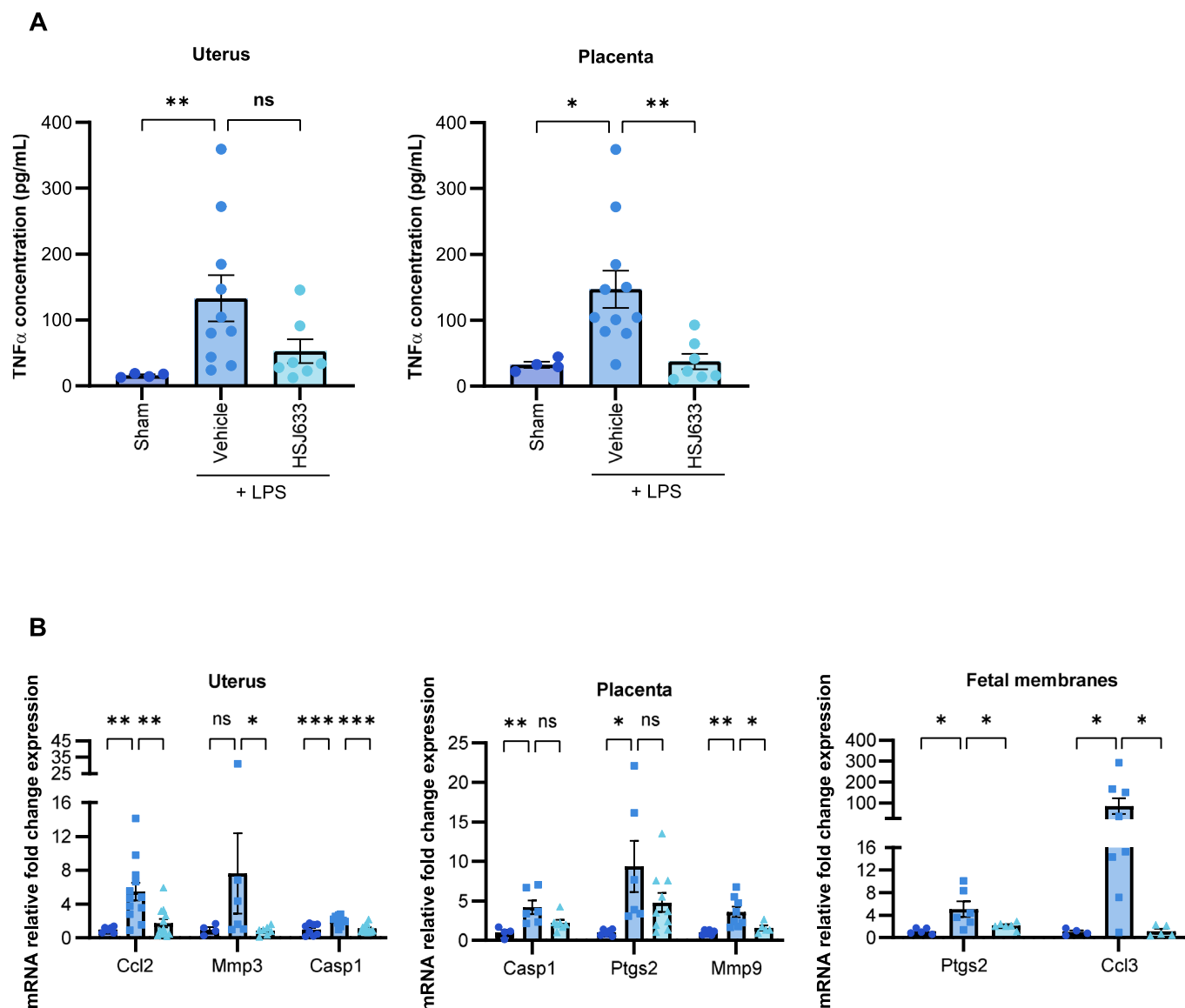


Figure EV2. Propylactic administration of HSJ633 reduces inflammation in reproductive tissues.

(A) TNF- α concentrations (quantified by ELISA) in the uterus and placenta on GD17.25. Values are mean $\pm$ SEM of 4–10 dams per group and one-way ANOVA with Dunnett's multiple comparisons test compared to LPS + vehicle group was performed for the placenta. Values are mean $\pm$ SEM of 4–11 dams per group and Kruskal-Wallis test with Dunn's multiple comparisons test compared to the LPS + vehicle group was performed for the uterus. * P < 0.05, ** P < 0.01. (B) mRNA expression on GD17.25 in the uterus, placenta, and fetal membranes of various genes involved in PTB (normalized to Actb). Values are mean $\pm$ SEM of 4–12 dams per group for the uterus, 4–10 dams per group in the placenta and 4–8 dams per group for fetal membranes. * P < 0.05, ** P < 0.01, *** P < 0.001.

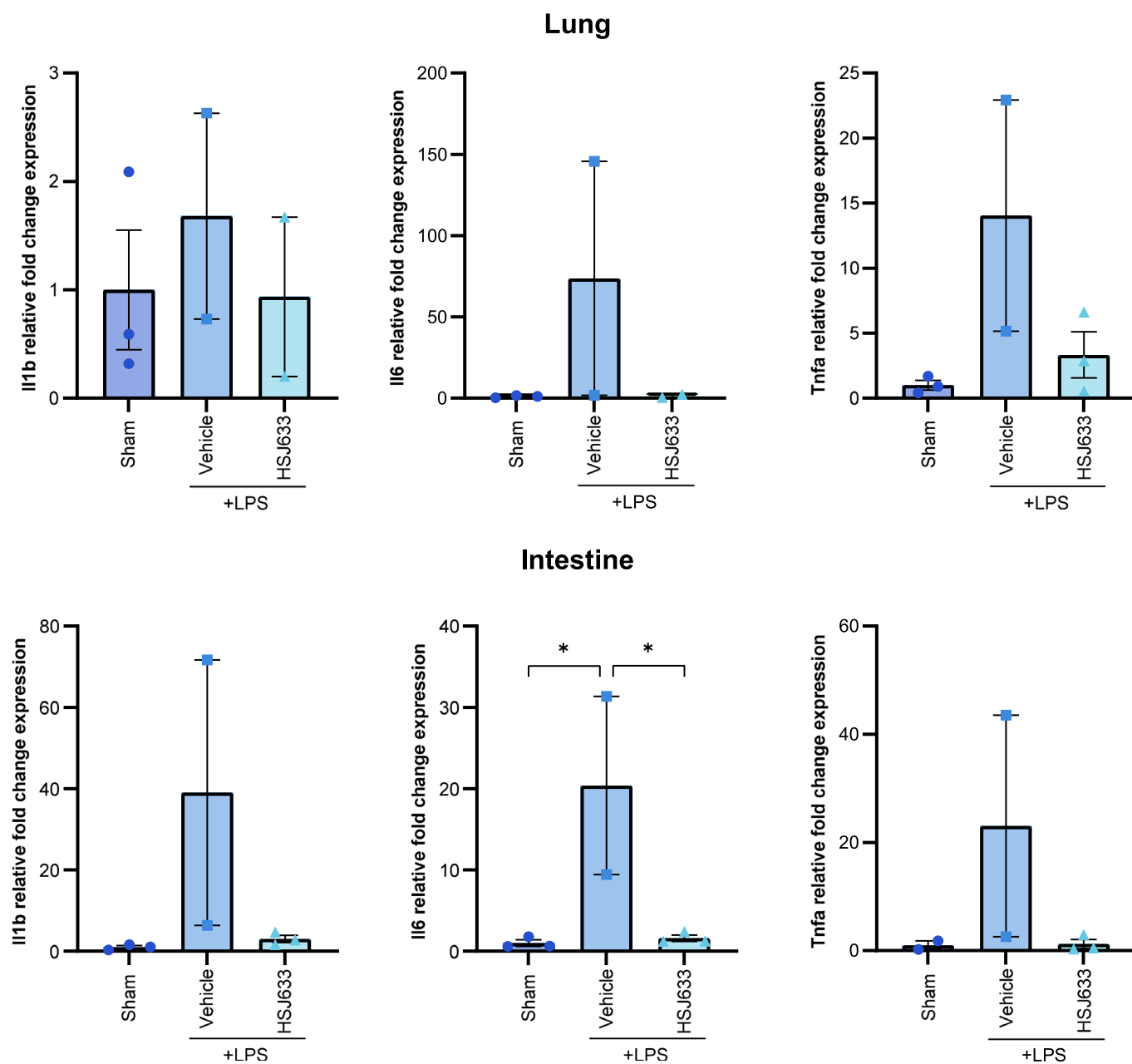


Figure EV3. LPS-induced inflammatory cytokines in fetal organs.

mRNA expression (determined by RT-qPCR) of IL-1 β , IL-6 and TNF- α in murine lung and intestine collected on GD17.25 after LPS induction (normalized to Actb). Values are mean $\pm$ SEM of 2-5 samples per group. One-way ANOVA with Dunnett's multiple comparisons test compared to LPS + vehicle group. * $P < 0.05$.

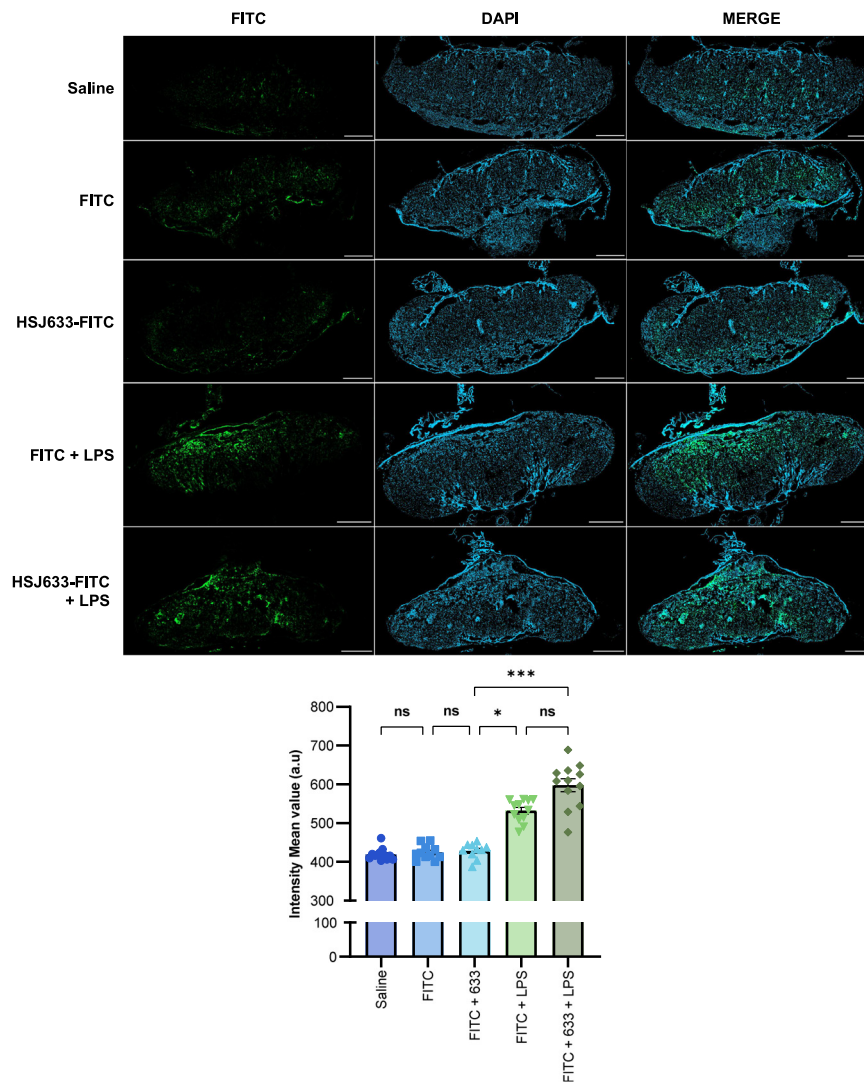
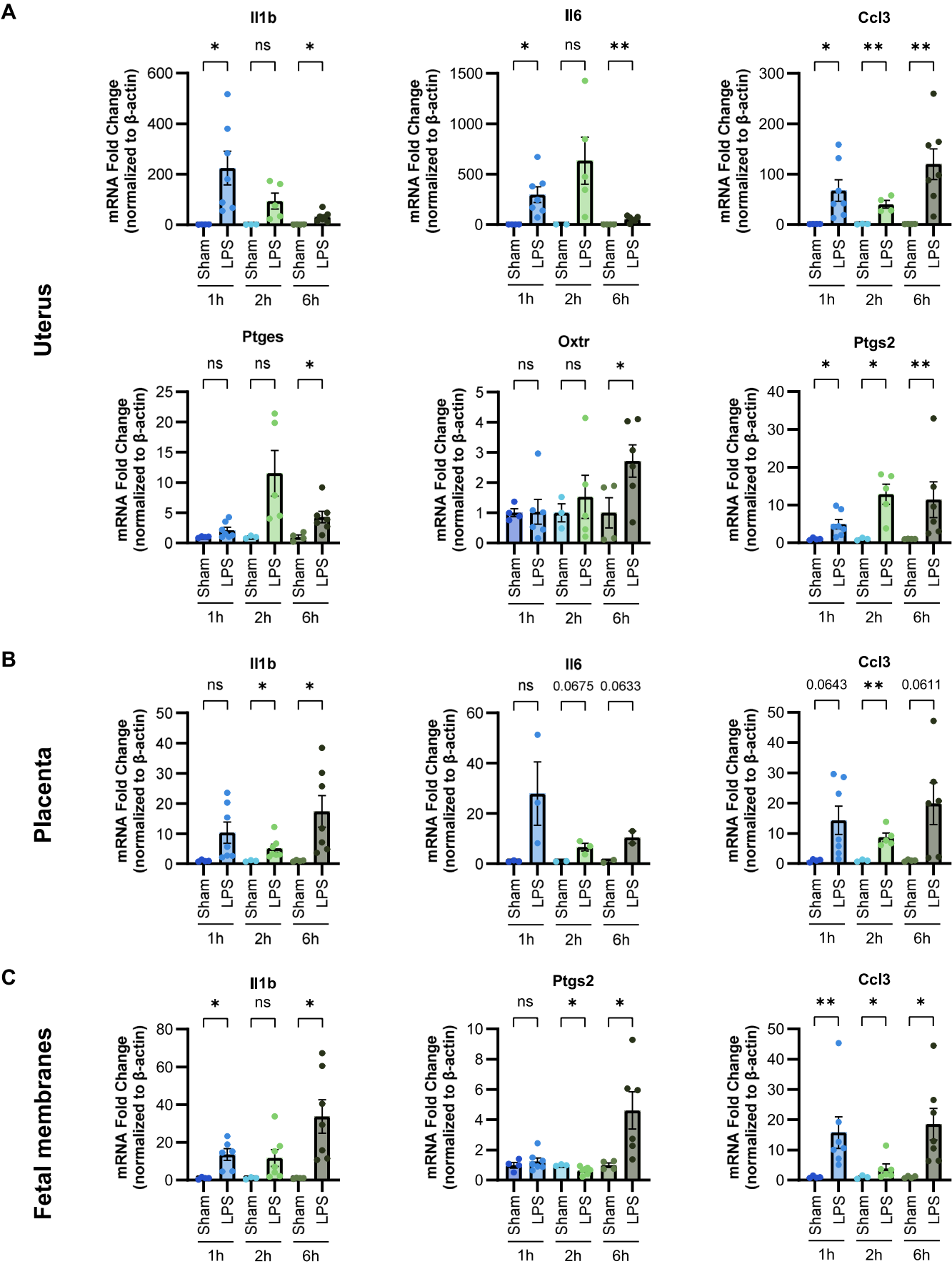


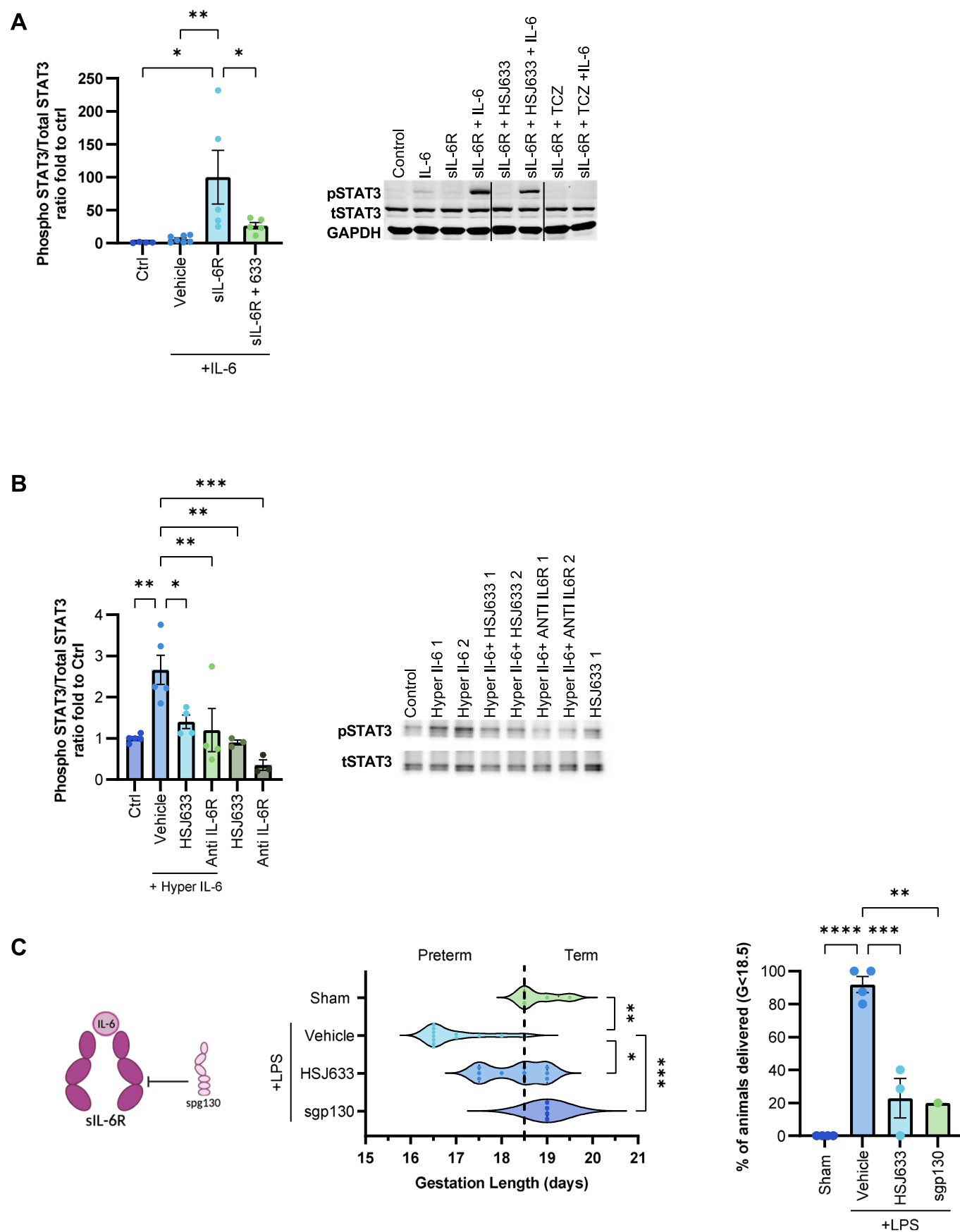
Figure EV4. Distribution of HSJ633 to fetal placental compartment.

Biodistribution of prophylactic HSJ633-FITC 4 h post subcutaneous injection in pregnant mice on GD17 in the placenta. Green fluorescence was quantified. Values are mean $\pm$ SEM of 11–12 samples per group. 1000 μ m scale bars. * P < 0.05, *** P < 0.001 by the Kruskal–Wallis test with Dunn’s multiple comparisons test compared to the LPS + vehicle group.



◀ Figure EV5. LPS induces an inflammatory response as early as 1 h post-administration in gestational tissues.

(A–C) mRNA expression (determined by RT-qPCR) of IL-1 β , IL-6, Ccl3, Ptgs2, Ptges, and Oxtr in the uterus, placenta, and fetal membranes collected 1 h, 2 h and 6 h after LPS injection on GD16 (normalized to Actb). Values are mean $\pm$ SEM of 3–7 samples per group. Unpaired *T* test compared to sham group at each time point with Mann-Whitney comparison when data were not distributed normally. **P* < 0.05, ***P* < 0.01.



◀ **Figure EV6. Importance of soluble IL-6R in human amniotic epithelial cells and in Ba/F3-mgp130-IL-6R α IL-6-triggered signaling and in LPS-induced PTB.**

(A) Amniotic epithelial cells were stimulated with IL-6 and/or sIL-6R (100 ng/mL for both) in absence or presence of HSJ633 (1 μ M), TCZ (3.44 μ M), or vehicle. Membranes were blotted for phospho-STAT3 and normalized to total STAT3. Representative Western blot gel on the right and compiled histogram data on the left. Values are mean $\pm$ SEM of 4–7 samples per group. One-way ANOVA with Dunnett's multiple comparisons test compared to sIL-6R + IL-6 group. (B) Quantification of STAT3 activation in Ba/F3-mgp130-IL-6R α treated with Hyper IL-6 (100 ng/mL), HSJ633 (0.1 mM) and Anti-IL-6R (200 μ g/mL). Representative Western blot displaying the levels of STAT3 in Ba/F3-mgp130-IL-6R α cells treated with hyper IL-6. Quantification of STAT3 activation presented as mean $\pm$ SEM of 3–5 samples per group. Densitometric analysis of the bands indicates a significant reduction in STAT3 activation in HSJ633+Hyper IL-6-treated cells relative to Hyper IL-6. One-way ANOVA with Dunnett's multiple comparisons test compared to Hyper IL-6. * P < 0.05, ** P < 0.01, *** P < 0.001. (C) Gestational length (left panel), data are presented as mean $\pm$ SEM of 4–12 dams per group and Kruskal–Wallis test with Dunn's multiple comparisons test compared to the LPS + vehicle group for gestation length. Rate of prematurity (born on GD < 18.5; right panel) in mice treated with soluble gp130 (sgp130) injected s.c. on GD16. Data are presented as mean $\pm$ SEM of 1–4 different experiments with 2 to 8 dams per group per experiment. One-way ANOVA with Dunnett's multiple comparisons test compared to the LPS + vehicle group. * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001. TCZ Tocilizumab.