Sup Figure 1

# WT/TLR3-KO CONTROL





## TLR3-KO MHV 68





Sup Figure 1c



E11.5

E17.5



Phase

Sup Figure 3

## a PBS + LPS



## **b** MHV68 + PBS



### c MHV68+LPS



Sup Figure 4



#### SUPPLEMENTARY MATERIAL

#### **Supplementary Figure 1**

**Effect of MHV-68 infection on fetal development**. Fetal and placental samples from wt and TLR-3KO pregnant mice infected with murine herpes virus 68 (MHV-68) at E8.5 were collected at day E11.5. (a) Control fetus and placenta from WT and TLR3 KO mice treated with PBS. Note the normal development of the fetus\* (b) fetus and placenta from TLR3 KO pregnant mice infected with MHV-68. Note the growth retardation of the fetus compared to the control. (\* Fetuses). c) Fetal weight of fetuses from WT and TLR3 KO mothers at 11.5 and 17.5 days post conception. Note the significant decrease in weigh of TLR3 KO fetuses from MHV-68 infected mothers.

\* P<0.05. Bars show median  $\pm$ SEM. Representative figure of six animals per group and three independent experiments.

#### **Supplementary Figure 2**

**Role of TLR-2 in MHV-68 replication in trophoblast cells**. First trimester trophoblast cells deficient of TLR-2 (TLR-2 DN) or TLR-1 (TLR-1 DN) were infected with GFP-labeled MHV-68, supernatant from these cultures were collected 48 hours after infection and transferred to new cultures of wild type first trimester trophoblast cells. (a) Wt trophoblast culture with supernatant from trophoblast infected with GFP labeled MHV-68 (b) Wt trophoblast culture with supernatant from TLR-2 DN trophoblast infected with GFP labeled MHV-68. (c) Wt trophoblast culture with supernatant from TLR-2 DN trophoblast infected with GFP labeled MHV-68. magnification, X20. White arrows indicate the presence of infected cells. n= 3 samples per group. Figures are representative of three independent experiments.

#### **Supplementary Figure 3**

MHV-68 viral infection sensitizes to LPS treatment. Wt mice were infected with either MHV-68 (1X10<sup>6)</sup> or PBS at E8.5 followed by a single dose of LPS (20ug/kg) or PBS at E15. (a)Control group where pregnancy mice received PBS followed by LPS. No changes are observed in the vagina. (b) Pregnant mice infected with MHV-68 followed by PBS. No macroscopic changes were observed, similar as the control. (c) Pregnant mice infected with MHV-68 followed by LPS. Note the presence of vaginal bleeding and dilation associated with parturition. Figures are representative of three independent experiments. n=6 mice per group.

#### **Supplementary Figure 4**

**MHV-68 infection sensitizes to LPS induced preterm labor.** Groups are similar as in Supp Figure 3. While normal gestational sacs are observed in the control group and in the animals infected with MHV68 followed by PBS treatment; fetal death and preterm delivery is observed in animals infected with MHV68 followed by LPS treatment. Representative figures of at least 6 mice per group.