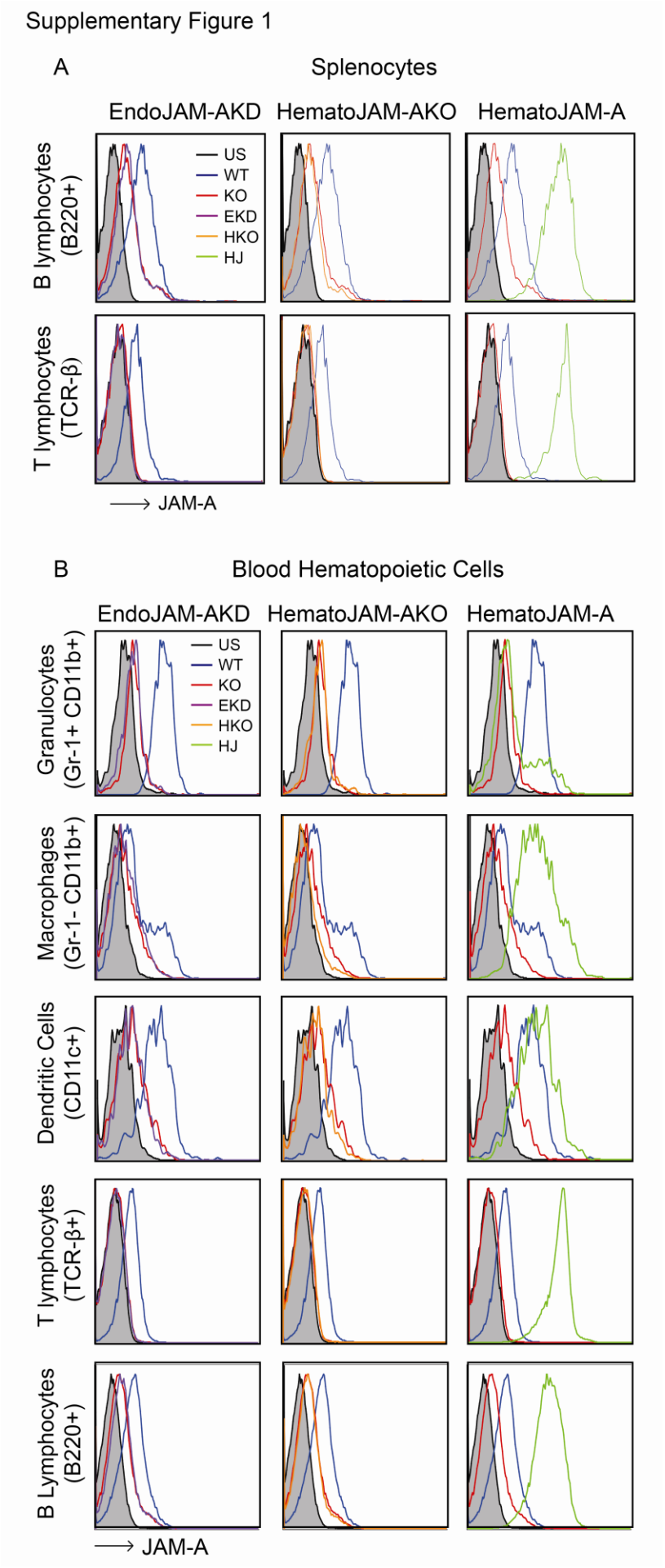
Supplementary Figure 1. JAM-A expression in circulating murine hematopoietic cells and splenocytes. (A) Spleens were excised from 6- to 8-week-old mice, and splenocytes were stained for various hematopoietic cell markers. B and T lymphocytes were defined as the populations that were $B220^+$ and TCR-β^+, respectively. Each set of histograms includes data from unstained, WT, and JAM-AKO cells along with the indicated genotype. WT = Wild-type (JAM-A f/f), KO = JAM-AKO, EKD = EndoJAM-AKD, HKO = HematoJAM-AKO, HJ = HematoJAM-A. (B) Blood was collected retroorbitally from 6- to 8-week-old mice, and JAM-A expression on leukocytes was quantified using flow cytometry. Granulocytes, macrophages, dendritic cells, B lymphocytes, and T lymphocytes were defined as the populations that were Gr-1^+, $CD11b^+$, $Cd11c^+$, TCR-β^+, and $B220^+$, respectively. Each set of histograms includes data from unstained, WT, and JAM-AKO cells along with the indicated genotype. WT = Wild-type (JAM-A f/f), KO = JAM-AKO, EKD = EndoJAM-AKD, HKO = HematoJAM-AKO, HJ = HematoJAM-A.

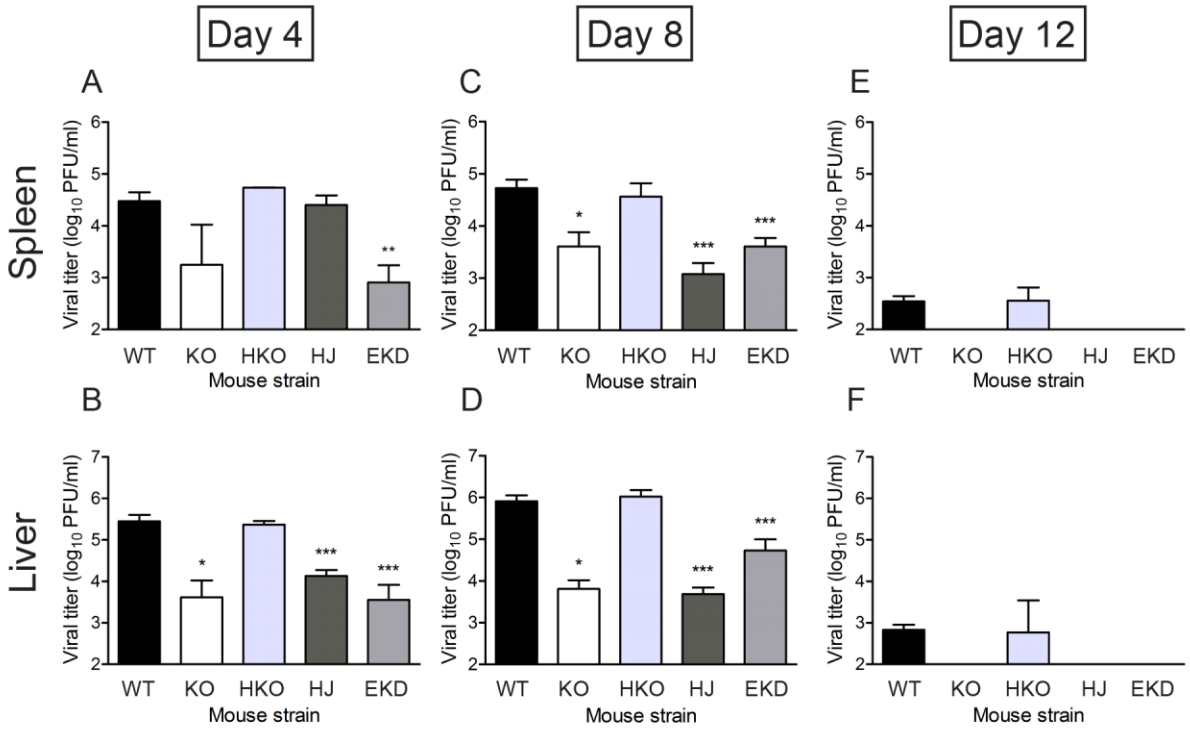
Supplementary Figure 2. Reovirus dissemination to the liver and spleen requires endothelial JAM-A. Newborn (2-3 d) mice were inoculated perorally with reovirus strain T1L at 1000 PFU per mouse. At 4, 8, and 12 d post-inoculation, spleen and liver were excised for determination of viral titer by plaque assay. Results are presented as mean viral titer. Error bars indicate standard deviation. For each time point and mouse strain, three to thirteen mice were used. WT = Wild-type (JAM-A f/f), KO = JAM-AKO, EKD = EndoJAM-AKD, HKO = HematoJAM-AKO, HJ = HematoJAM-A. *, $P < 0.05$, **, $P < 0.005$, ***, $P < 0.001$ by Student's *t* test.

Supplementary Figure 3. Reovirus does not bind to or infect splenic hematopoietic cells. Newborn (2-3 d) wild-type mice were inoculated perorally with either PBS or reovirus strain T1L at 5×10^6 PFU per mouse. At 8 d post-inoculation, hematopoietic cells from the spleen were isolated and stained with antibodies specific for hematopoietic cell subsets and reovirus-specific polyclonal antiserum using non-permeabilizing (to detect binding) or permeabilizing (to detect infection) conditions. The percentage of infected cells within each cell population was determined using flow cytometry.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3