

Supplementary Figure 1. TLR8 signaling negatively regulates TLR7-mediated antiviral immunity and facilitates WNV infection in BMDCs. (A) Flow cytometric analysis of TLR7 expression in WT and $Tlr8^{-/-}$ BMDCs infected with WNV (MOI = 5) for 24 hr (n = 3 mice per group). WT (grey outline), $Tlr8^{-/-}$ (black outline), secondary only IgG isotype control (grey filled) mice. Inset: Mean fluorescent intensity (MFI). (B) qPCR analysis of gene expression of $Ifn-\beta$ in BMDCs after stimulated with TLR7 ligand CL264 (5 μg/ml) *in vitro*. (C) qPCR analysis of gene expression of $Ifn-\beta$ in BMDCs following infection of WNV or CHIKV (MOI = 5) for 24 hr. (D) Mean pixel intensity of WNV antigen (Ag) arbitrary units (a.u.) per 3 x 10⁵ cells/well from immunocytochemistry images (Figure 2J) were quantified using ImageJ (version 1.48) and an Axiostar Plus light microscope (n = 3 mice per group). (E) Ratio of MFI of WNV-E antigen / DAPI+ cells between WT and $Tlr8^{-/-}$ BMDCs infected with WNV (MOI = 5) for 24 hr (n = 3 mice per group). All qPCR assays were performed three independent times, and the flow cytometric analyses were performed two times. Data were analyzed using a two-tailed, Student's t-test (* denotes p < 0.05, ± 1 SEM).