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Supplemental Information

Protein Kinase R Contributes to Immunity against Specific Viruses by Regulating Interferon mRNA Integrity

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Supplemental Information

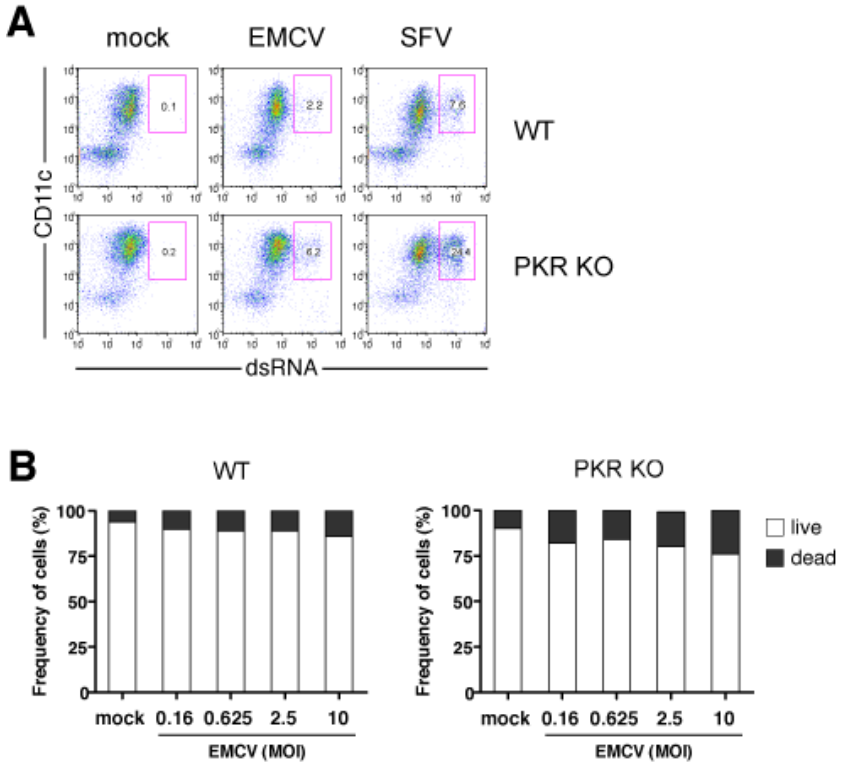

Figure S1. Failure of PKR$^{-/-}$ BM-DCs to produce IFN-α/β does not correlate with reduced levels of viral RNA or increased cell death. (A) BM-DCs were infected with EMCV (MOI=10), SFV (MOI=5) or left untreated (mock) and stained with a mAb (K1) specific for dsRNA before analysis by flow cytometry. Dot plots show cells expressing the DC lineage-specific marker CD11c and dsRNA. The frequency of CD11c/dsRNA double positive cells is shown inside the rectangular gates. (B) Wild-type (left panel) and PKR$^{-/-}$ BM-DCs (right panel) were stained with a live/dead cell discriminating dye after overnight incubation in the absence or presence of EMCV (MOI=10) and analyzed by flow cytometry. Histogram shown represent percentage of dead cells (filled section of bar) and live cells (open section of bar) for each treatment. Data in A and B are representative of two independent experiments.

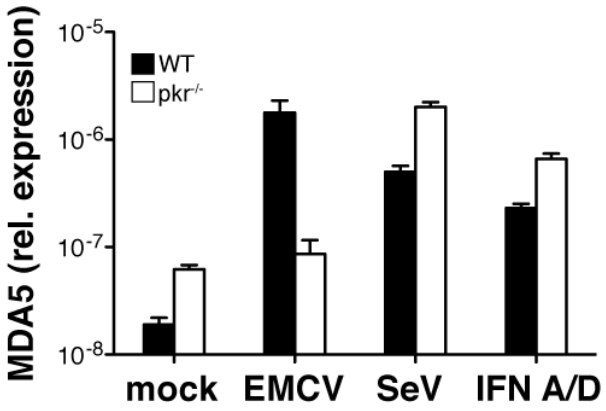

Figure S2. PKR$^{-/-}$ BM-DCs fail to up-regulate MDA5 mRNA after EMCV infection.

BM-DCs ($5x10^5$/well) were infected with EMCV (MOI=10), SeV (MOI=1) or treated with IFN A/D for 12 hours. MDA5 mRNA was analyzed by quantitative PCR and levels were calculated relative to 18s rRNA. Data are the mean ± SD of triplicate wells and are representative of three independent experiments with similar results.

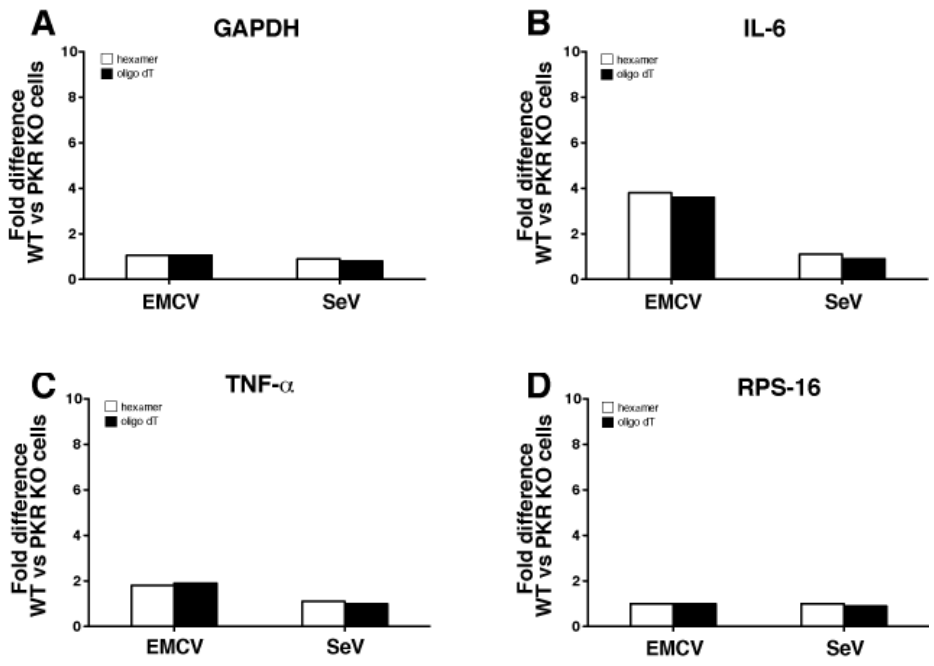

Figure S3. Quantitative PCR analysis of adenylation status of various inducible and constitutive genes following viral infection. BM-DCs were infected with EMCV (MOI=10) or SeV (Moi=1) for 12 hrs and 6 hrs respectively. cDNA for GAPDH (A), IL-6 (B), TNF-α (C), and RPS-16 (D) was generated from total RNA using random hexamer (open bars) or oligo dT (filled bars) nucleotides. The relative expression of each gene in $PKR^{+/+}$ versus $PKR^{-/-}$ cells was calculated from the Ct values and converted into fold difference using the formula $2^{(-\Delta Ct)}$. Data are representative of two independent experiments.