Supplemental Figures

Figure S1. Female C57BL/6 mice were infected i.v. with 10^6 pfu rVV-FL-OVA. At 3 or 14 days post-infection, mice were sacrificed and spleens were taken for analysis. For half the samples, the whole spleen was freeze-thawed 3x, then Dounce homogenized, filtered through a 40um strainer, and sonicated. For half the samples, the whole spleen was incubated in 1 mg/ml collagenase D for 60 minutes, then ground through a 40um filter and incubated in ACK lysis buffer for 10 minutes to lyse red blood cells. The remaining cells were spun down and resuspended in 5ml HBSS, then freeze-thawed 3x, then homogenized, filtered and sonicated. All homogenized samples were subjected to a series of 10-fold dilutions and titered in triplicate on TK-minus cell monolayers. Dotted lines indicate the limit of detection.

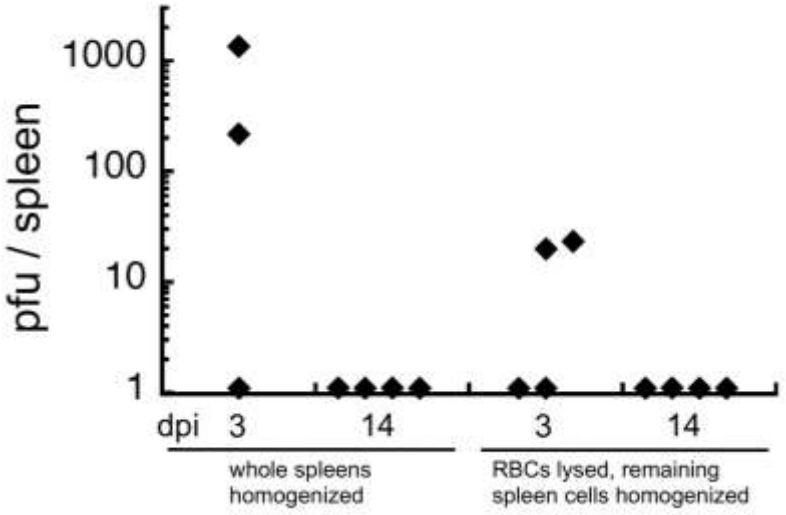

Figure S2.

Female C57BL/6 mice (n=4) were infected i.v. with 10^6 pfu of rVACV-OVA. At each day post-infection, ovaries were extracted and frozen in –80°C. Tissue was ground in a mortar and pestle over liquid nitrogen, and RNA was extracted and transcribed into cDNA. OVA primers were used to detect the presence of actively replicating virus. Limit of detection was 10 copies of cDNA in a volume representing 10% of total ovary tissue.

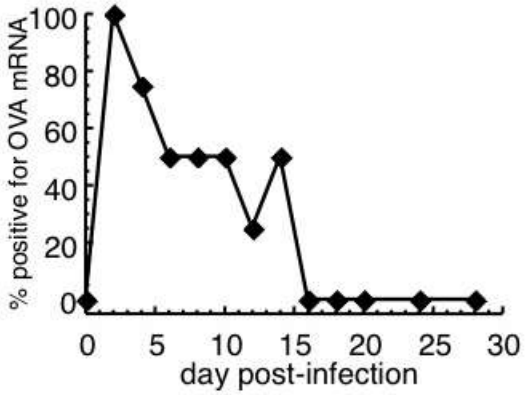

Figure S3

WT3 cells were infected with rVACV that had been treated with psoralen and UVA for the times indicated. β-gal was either driven by the early/late promoter p7.5 (red) or by the late promoter p11 with (white) or without AraC (blue). β-gal production was measured by incubating with a β-gal substrate that contained 0.125% Igepal and chloropheno red-β□D-galactopyranoside. A_{570} was determined with an MRX plate reader.

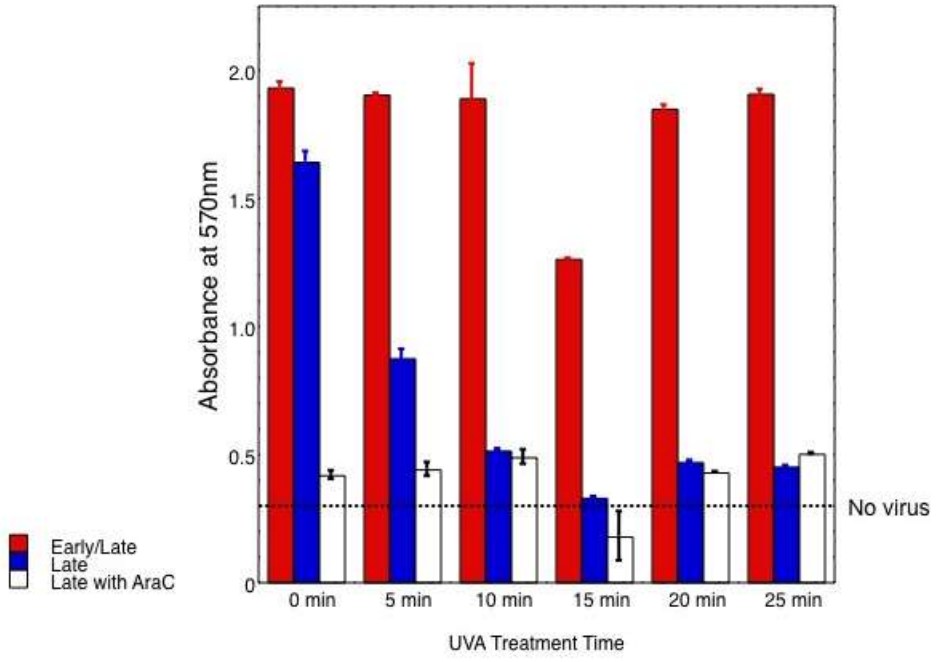

Figure S4: gp91*phox*$^{-/-}$ mice do not show a defect in cross presentation of antigen following immunization with β2M$^{-/-}$ cells infected with rVACV expressing OVA FL.

gp91*phox*$^{-/-}$ or C57BL/6 mice were immunized with β2M$^{-/-}$ cells infected with rVACV expressing OVA FL at times indicated. OT-1.SJL T cells were labeled with CFDA-SE and transferred i.v. into previously immunized mice. 3 days after transfer, spleens were removed and analyzed for CFDA-SE dilution. Histograms were gated on CD45.1^{+} T_{CD8+} and are representative of two individual mice. Gates represent the percentage of CD45.1^{+} T_{CD8+} cells that proliferated. Numbers represent the standard error of percent CD45.1+ T_{CD8+} proliferation.