OMTM, Volume 17

## **Supplemental Information**

## Nanoplasmid Vectors Co-expressing Innate Immune

### **Agonists Enhance DNA Vaccines for Venezuelan**

## **Equine Encephalitis Virus and Ebola Virus**

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# Figure S1. Histogram Plots of GP Expression from COS-7 Cells Transfected with the Various VEEV GP Expressing DNA Vaccines

Representative histogram plots were generated for COS-7 cells transfected with 50 ng empty vector, pWRG/VEEV, or the individual Nanoplasmid vaccines. For these representative plots, the percentage of GP expressing cells were determined by staining for VEEV E1 surface expression with monoclonal antibody 3B2A-9. These data are representative of that shown in Figures 1A, C, E.

### Ratio IgG2a:IgG1



#### Figure S2. isRNA Expressing VEEV Nanoplasmids Favor an IgG2a Humoral Response

Female BALB/c mice were vaccinated with 5  $\mu$ g of pWRG/VEEV or the various VEEV Nanoplasmids on days 0 and 21 by IM injection. VEEV GP-specific antibody responses were analyzed 21 days after the second vaccination by ELISA. The ratio of VEEV-specific IgG2a:IgG1 antibody titers are shown. Mice that were euthanized or succumbed to disease following 10<sup>4</sup> PFU VEEV aerosol challenge are shown in red. Data are the group mean averages +/- SEM.

## Ratio IgG2a/IgG1





Female BALB/c mice were vaccinated with 25 µg of pWRG/EBOV or the various EBOV Nanoplasmids on days 0 and 21 by IM injection. EBOV GP-specific antibody responses were analyzed 21 days after the second vaccination by ELISA. The ratio of EBOV-specific IgG2a:IgG1 antibody titers are shown. Data are the group mean averages +/-SEM.