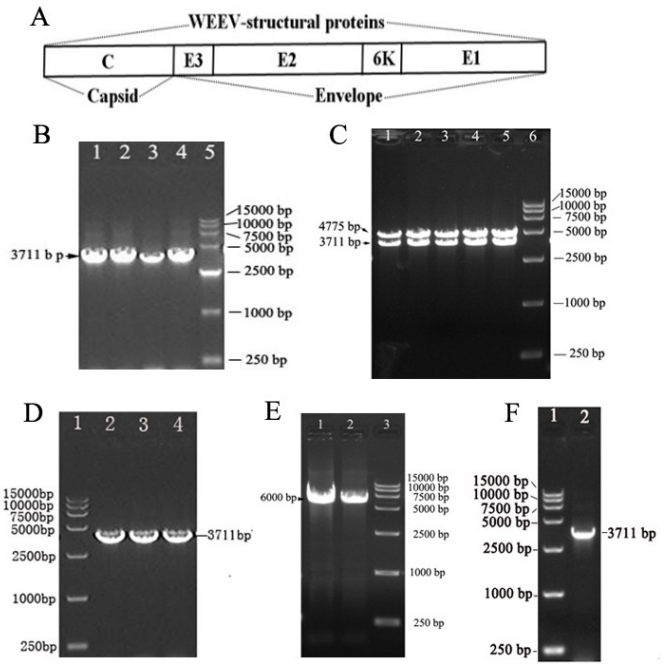

Figure 1. Plasmid construction and generation of recombinant Baculoviruses.

A. Schematic representation of the WEEV structural cassette. B. The 3711 bp target fragments (*c-e3-e2-6k-e1*) were obtained by using PCR. C. The confirmation of the pfastBac^{TM}1-*c-e3-e2-6k-e1* plasmid. The digestion results showed that the 3711 bp and 4775 bp fragments were obtained. D. The PCR identification of the pfastBac^{TM}1-*c-e3-e2-6k-e1* plasmid. E. The confirmation of the recombinant bacmid DNA. The about 6000 bp PCR segments were exhibited. F. The evaluation of the recombinant bacmid DNA. The 3711 bp PCR segments were displayed.

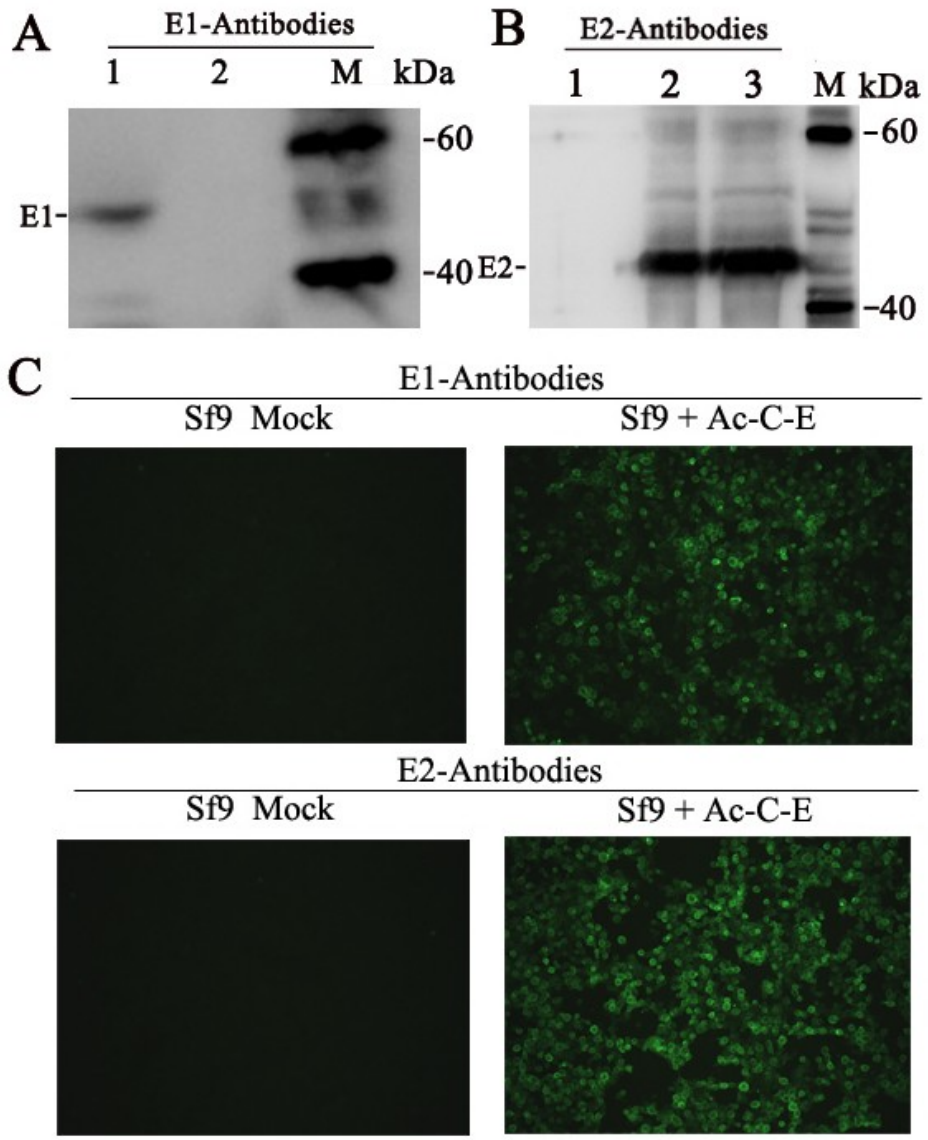

Figure 2. WEEV structural cassette expression and analysis.

The structural proteins, including capsid (C) and envelope (E3, E2, 6K and E1) were expressed in Sf9 cells. A. Western blot analysis of WEEV E1 protein expression. The purified VLPs were treated for Western blot analysis as described in Materials and Methods. 1 lane: VLPs; 2 lane: Mock. B. Analysis of WEEV E2 protein expression. 1 lane: Mock; 2, 3 lane: VLPs. C. Detection of E1 and E2 proteins in Ac-C-E-infected Sf9 cells by immunofluorescence assay. Sf9 cells were infected with Ac-C-E and subjected to immunostaining using E1 and E2 antibody, respectively. Cells were analyzed using fluorescent microscopy (Magnifications: 10X10).

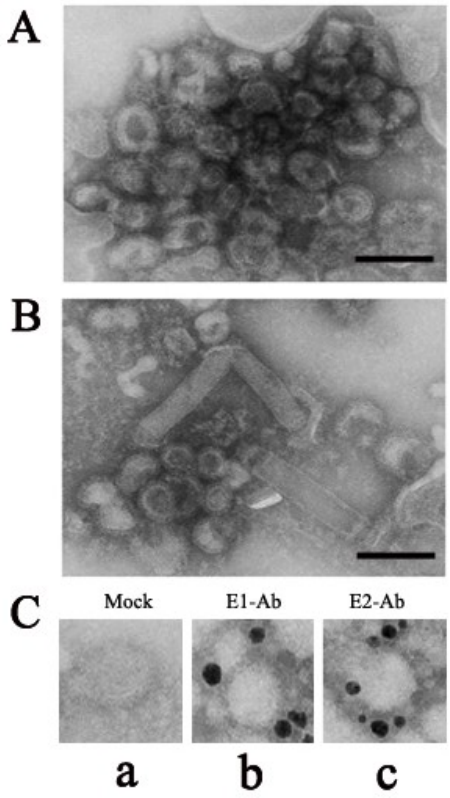

Figure 3. Negative staining and immunogold-label of VLPs.

VLP preparations were stained with 1% phosphotungstic acid and analyzed using a transmission electron microscope. A. Gradient-purified VLPs. B. The supernatants of Ac-C-E-infected Sf9 cells. Bar, 100 nm. C. Analysis of VLPs using immunoelectron microscope. Gradient-purified particles were absorbed onto grids and processed for immuno-gold labeling as described in Material and Methods. (a). Mock. (b). VLPs were treated with an anti-E1 monoclonal antibody and counterstained with colloidal protein A-gold (PAG) particles (c). VLPs were probed with anti-E2 antibody and counterstained as described above.

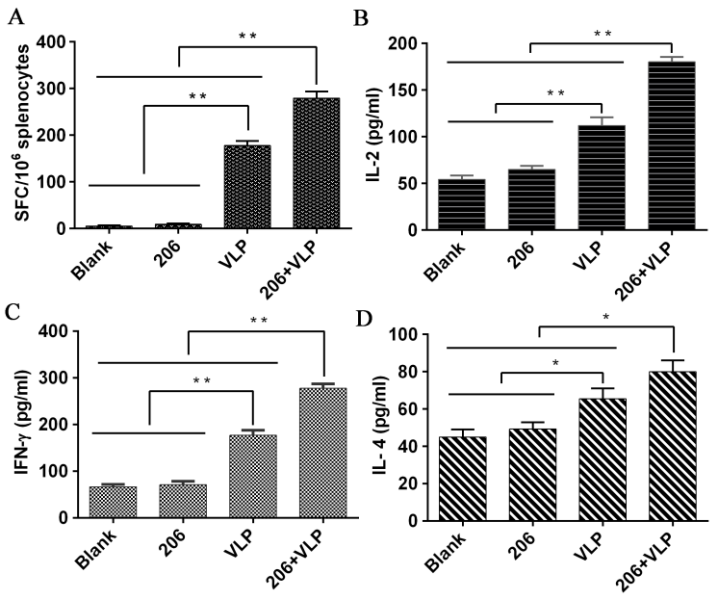

Figure 4. ELISpot analysis of IFN-γ secretion and the cytokine production of splenocytes from immunized mice.

(A) The immune effects of WEEV VLP were assessed by IFN- γ ELISpot analysis. Data represented the mean of spot-forming cells (SFCs) per million splenocytes from five mice/group with the standard deviation (SD). Statistical differences between groups were determined, and significant *p* values between vaccinated groups are shown as ** $p < 0.01$. (B) IL-2, (C) IFN-γ and (D) IL-4 cytokines from supernatants of splenocytes stimulated by WEEV VLPs were quantified by ELISA. IFN- γ, IL-2 and IL-4 levels were detected and data were shown as means ± SD. Statistical differences between groups are shown as $p^* < 0.05$ or ** $p < 0.01$. The data represents the means (M±SD) of 5 mice per group.

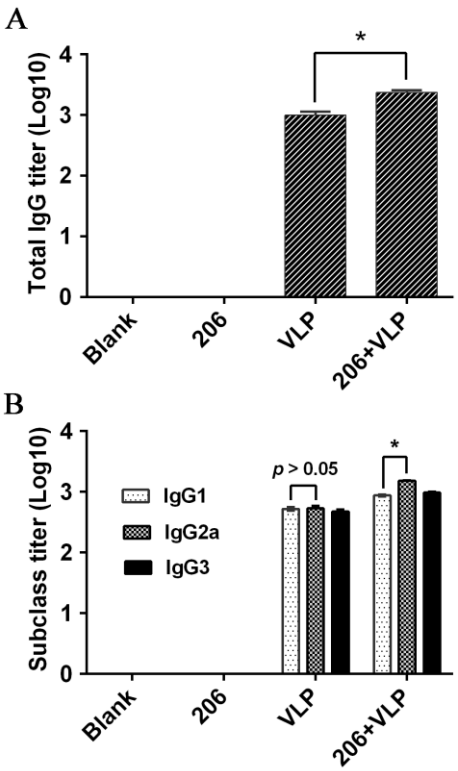

Figure 5. Analysis of antibody in serum from vaccinated mice.

(A) and (B) showed the total IgG and IgG subclasses (IgG1, IgG2a and IgG3) responses to WEEV E2 proteins, respectively. The antibody level against E2 proteins was determined by ELISA assay. The results are the mean value (Log10 value) of each group of mice (n = 5) ± SD, and the significant differences were expressed as * p <0.05. The data indicated that the mice immunized with 206-VLP in their serum generated more strong antibody response against the WEEV E2 protein than the VLP-immunized mice did, and the IgG2a titers were markedly higher than IgG1 ones in serum from 206-VLPs-immunized group.

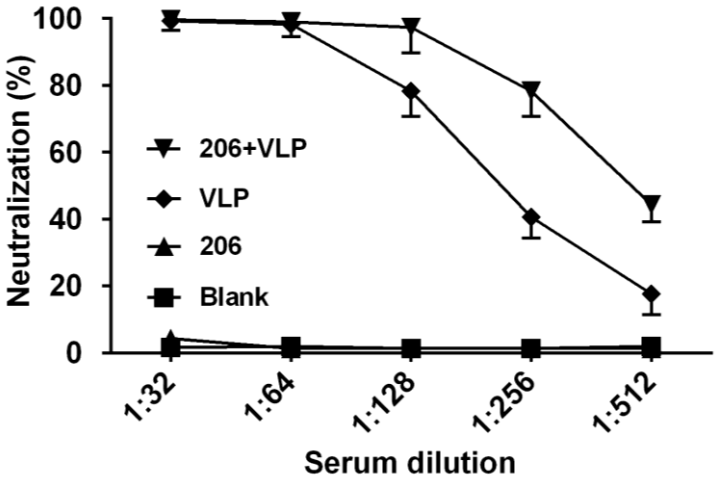

Figure 6. Neutralization assay of WEEV pseudovirus against sera from immunized mice.

Serum samples from mice (5 mice per group) fourteen days after the second immunization were at 1:32, 1:64, 1:128, 1:256 and 1:512 dilutions, and each dilution was incubated with 500 IU of WEEV pseudovirus, respectively. The results were analyzed as described in Materials and Methods. The values are mean ± SD (n=5).