## Supplemental Materials

### Facility

The live viruses involved in this study were performed in the biosafety level 3 facility of the Wuhan Institute of Virology, University of Chinese Academy of Sciences (CAS).

### Cell culture and virus isolation

Porcine alveolar macrophages (PAMs) were prepared by bronchoalveolar lavage and grown in RPMI 1640 (Gibco, USA) supplemented with 10% fetal bovine serum (Sigma, USA), penicillin (100 U/mL), streptomycin (100 μg/mL) and amphotericin B (250 ng/mL) (Beyotime Biotechnology, China) at 37 ℃ with 5%$CO_2$ (1).

The samples including blood and tissues were collected from farms and slaughterhouses during the surveillance. Positive samples detected by the qPCR assay were homogenized and inoculated into PAM cells. After 4 to 7 days post-inoculation, when the cells showed a cytopathic effect, the supernatant was collected to further identify virus propagation by using the qPCR assay (2). Virus stocks were stored at −80°C for use.

### HAD assay

$4\times10^4$ cells/well of primary PAMs were cultured in 96-well plates and infected with 10-fold diluted ASFVs. After 24 hours infection, 1% porcine erythrocyte cells washed and resuspended with PBS (Gibco, USA) were added into the 96-well plates. The phenomena of hemadsorption were observed over 7 days by a microscope. The $HAD_{50}$ was calculated by using the Reed and Muench method (3).

### Indirect immunoperoxidase test (IPT)

$6\times10^4$ cells/well of primary PAMs cells were inoculated into 96-well plates. The plates were infected at a MOI of 0.2 with the isolated ASF viruses incubated in a humidified atmosphere containing 5% $CO_2$ at 37℃ for 48 h. After incubation, the medium were removed and the ASFV-infected cells were fixed with a cold solution containing 4% polyformaldehyde at room temperature for 20 min. Finally the plates were washed five times with PBS and can be used directly or stored at -20℃.

The fixed ASFV plates were kept at room temperature for 30 minutes. Then 100 μL/well blocking solution (5% skim milk, 0.05% Tween 20, PBS, pH 7.4) was added into the fixed ASFV plates and incubated at 37℃ for 1 h. Positive, negative serum and samples to be confirmed diluted at 1/40 with blocking solutions were added 100 μL/well into the ASFV plates at 37℃ for 45 minutes on a plate shaker. The groups of PAM cells without ASFV infection incubated with the corresponding serum were used as the negative control. Four repeat wells for each group. The plates were washed five times with PBS for 5 minutes at 37℃ on a plate shaker. 100 μL/well of goat anti swine IgG H&L (HRP) secondary antibody (Biodragon Immunotechnologies, China) diluted at 1/1000 with blocking solutions were added into the ASFV plates at 37℃ for 45 minutes on a plate shaker. After washing five times with PBS, 50 μL of the AEC (3-amino-9-ethylcarbazole) peroxidase substrate (Yeasen Biotechnology, China) diluted in acetate buffer was added into each well of the plates and incubated at room temperature for 30 minutes. Then 100 μL/well distilled water was added to stop the reaction. The sera presenting an intensive red cytoplasmic color in the cells were considered as ASFV antibody positive and those with absence of red color were recorded as negative (4).

**Western blotting assay**

P30-Luc protein expressed by *E.coli* were mixed with 5×protein loading buffer and boiled at 98℃ for 10 min. Following separation by 10% SDS-PAGE gel, proteins were transferred on a polyvinylidene diflouride membranes (PVDF, Millipore, USA) blocked with 5% skim milk and incubated with positive serum and negative serum (1:1000) at room temperature for 1 h, respectively. After washing with PBST (containing 0.2% Tween20), the membrane was further incubated with HRP-conjugated goat anti-swine IgG secondary antibody (1:10000) at room temperature for 1 h (5). After washing with PBST, the membrane was visualized using a digital image system.

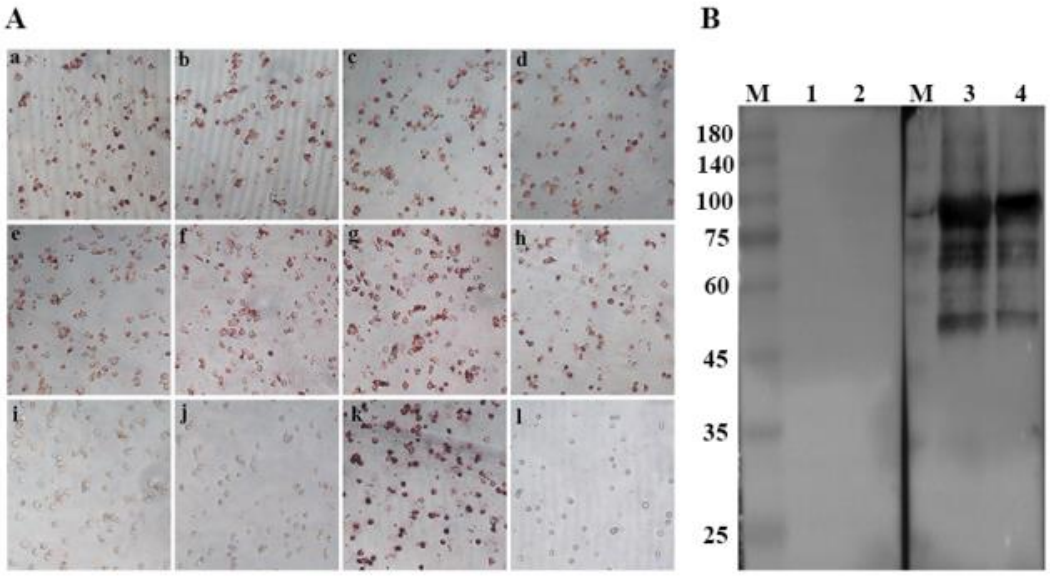


**Figure. S1.** Validation of the clinical swine serum samples by indirect immunoperoxidase test (A) and specific confirmation of recombinant p30-Luc with clinical swine serum samples by western blot (B). a, b, c, d, e, f, g, h, i, j represent the clinical swine sera samples that showed inconsistence results when tested by the LIPS assay and the commercial ELISA kit; k, a confirmed ASFV positive serum sample; l, an ASFV negative serum sample; the magnification of the objective is 10×. M, protein marker; 1.3 μg (lane 1) and 0.65 μg (lane 2) p30-Luc incubated with swine negative serum diluted at 1/1000; 1.3 μg (lane 3) and 0.65 μg (lane 4) p30-Luc incubated with swine positive serum diluted at 1/1000.

**References**


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