Applied Microbiology and Biotechnology

Generation and Functional Analysis of Single-chain Variable Fragments

(scFvs) Targeting the Nucleocapsid Protein of Porcine epidemic diarrhea

virus

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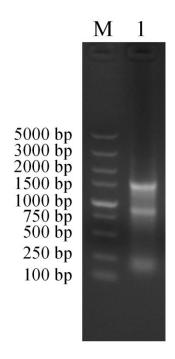


Fig. S1 Analysis of RNA with gel electrophoresis. Lane M, 5000-bp DNA ladder; lane 1, RNA extracted from the spleens of immunized pigs

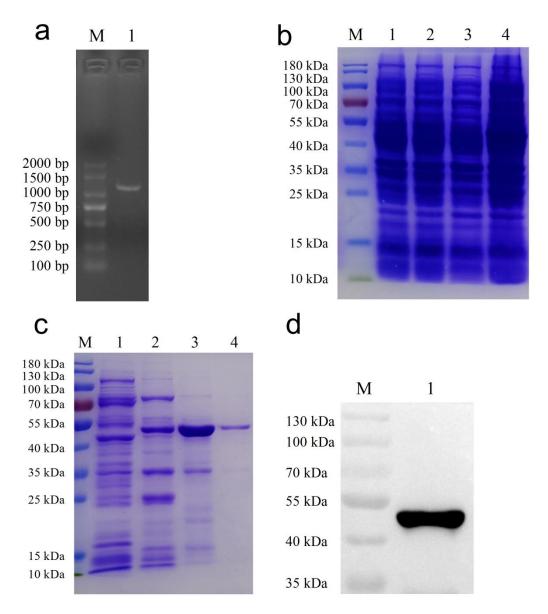


Fig. S2 Expression and purification of the recombinant TGEV N protein. **a** RT-PCR amplification of CDS region of TGEV N gene. Lane M, 2,000-bp DNA ladder; lane 1, PCR product of TGEV N gene. **b** SDS-PAGE analysis of proteins extracted from bacterial cell lysate. Lane M, protein molecular weight marker; lane 1, total cell lysate of uninduced *E. coli* containing pCold I; lane 2, total cell lysate of induced *E. coli* containing pCold I; lane 3, total cell lysate of uninduced *E. coli* containing pCold I-TGEV-N; lane 4, total cell lysate of induced *E. coli* containing pCold I-TGEV-N. **c** SDS-PAGE analysis of the purified protein. Lanes 1–4, purified TGEV N proteins eluted at different imidazole concentrations (50 mM, 100 mM, 250 mM, 500 mM, respectively). **d** Western blotting analysis of the purified protein. Lane 1, purified TGEV N protein

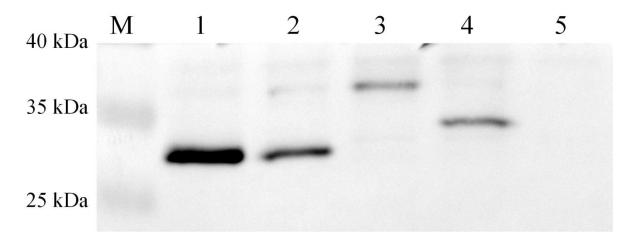


Fig. S3 Western blotting analysis of cell lysates. Lanes 1–5, lysates of Vero E6 cells transfected with pCMV-HA-ZW1-16, pCMV-HA-ZW3-21, pCMV-HA-ZW1-41, pCMV-HA-ZW4-16, or pCMV-HA, respectively; scFv expression was detected with western blotting

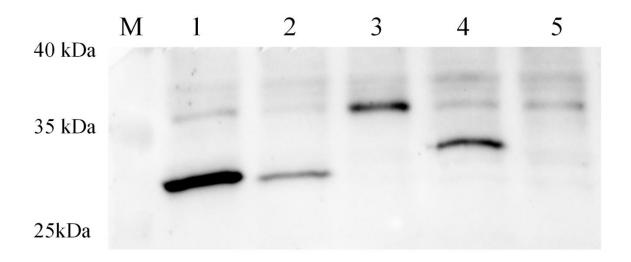


Fig. S4 Western blotting analysis of cell lysates. Lanes 1–5, lysates of IPEC-J2 cells transfected with pCMV-HA-ZW1-16, pCMV-HA-ZW3-21, pCMV-HA-ZW1-41, pCMV-HA-ZW4-16, or pCMV-HA, respectively; scFv expression was detected with western blotting

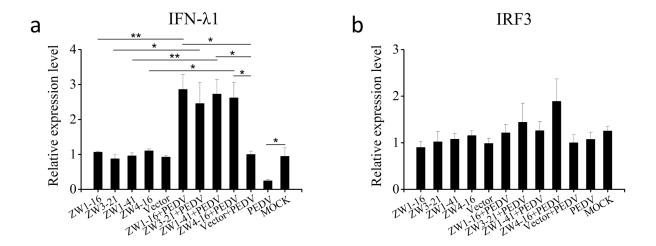


Fig. S5 Analysis of the relative gene expression of IFN- λ 1 and IRF3 by RT-qPCR. IPEC-J2 cells were transfected with pCMV-HA-scFv or pCMV-HA before PEDV infection. Untransfected infected IPEC-J2 cells and transfected uninfected IPEC-J2 cells were used as the control, and untransfected uninfected IPEC-J2 cells were used as the MOCK control. At 12 hpi, total RNA was isolated from the treated cells, and the transcriptional expression of IFN- λ 1 and IRF3 was detected with RT-qPCR. The housekeeping gene β-actin was used to normalize the individual samples. Values are the means \pm standard errors of the means (mean \pm SEM) of three independent experiments. *P < 0.05, **P < 0.01