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**Supplementary Data**

**Immunization of mice with vaccinia virus Tiantan strain yields antibodies cross-reactive with protective antigens of monkeypox virus**

**Lei Yang a,b,1, Yingshan Chen a,b,1, Sha Li a,b,1, Yuan Zhou a, Yongli Zhang a, Rongjuan Pei a, Xinwen Chen a,c,\*, Yun Wang a,\***

*a State Key Laboratory of Virology, Center for Biosafety Mega-Science, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan, 430071, China*

*b University of Chinese Academy of Sciences, Beijing, 100049, China*

*c Innovation Center for Pathogen Research, Guangzhou Laboratory, Guangzhou, 510320, China*

\* Corresponding authors.

*E-mail addresses*: wangyun@wh.iov.cn (Y. Wang), chen\_xinwen@gzlab.ac.cn (X. Chen)

1 Lei Yang, Yingshan Chen and Sha Li contributed equally to this work.

**Materials and methods**

**Plasmid production and bacterial expression**

The monkeypox orthologs of the VACV *A27L*, *A33R*, *B5R*, *D8L*, *H3L* and *L1R* genes are *A29L*, *A35R*, *B6R*, *E8L*, *H3* and *M1R*, respectively. The monkeypox genes were synthesized by BGI and the gene of VACV was amplified by PCR with corresponding primer synthesized by Sangon Biotech from genome of VTT. The protective antigens of monkeypox and VTT were tagged with HA at their 3′-end by PCR and then cloned into pGEX-KG vector for expression as glutathione S-transferase (GST) fusion proteins on their N terminus. The bacterial expression plasmids were transformed into expression host cells BL21 (DE3). Cells were grown in LB at 37 °C to a cell density of OD600 = 0.6–1 and induced with 1 mmol/L isopropyl-β-D-thio-galactoside (IPTG) (Sangon Biotech) for 2 h at 37 °C. The cells were harvested by centrifugation at 5000×*g* for 2 min and stored at −80 °C until purification.

**Western blot**

The bacterial cell pellet was chemically lysed with bacterial lysis buffer (Sangon Biotech), and total protein was subjected to a Western-blot assay. Six-week-old female BALB/c mice (n = 6) were intraperitoneally vaccinated with VTT of 5 × 105 plaque forming units (PFUs). At 21 days after the prime vaccination, mice received a boost inoculation. Mice were euthanized at Day 28 post vaccination and their sera were collected and pooled. The immunoblots were probed with anti-HA antibody (51064-2-AP, Proteintech), and the same amounts of total protein were probed with a serum mixture collected from pre-immune and immunized mice. A densitometry assay using ImageJ software was employed to quantitatively analyze the immunoblots to quantify the cross-reactivity.

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**Supplementary Fig. S1** Sequence alignment of protective antigens between VTT and monkeypox virus. **A** Sequence alignment between A27L of VTT and A29L of monkeypox virus. **B** Sequence alignment between A33R of VTT and A35R of monkeypox virus. **C** Sequence alignment between B5R of VTT and B6R of monkeypox virus. **D** Sequence alignment between D8L of VTT and E8L of monkeypox virus. **E** Sequence alignment between H3 of VTT and H3L of monkeypox virus. **F** Sequence alignment between L1R of VTT and M1R of monkeypox virus.