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Electronic Supplementary Material

Improving Cross-Protection against Influenza Virus Using Recombinant Vaccinia Vaccine Expressing NP and M2 Ectodomain Tandem Repeats

Wenling Wang[1]✉, Baoying Huang[1], Xiuping Wang[1], Wenjie Tan[1], Li Ruan[1]✉

1. NHC Key Laboratory of Biosafety, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China

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Materials and Methods

Identification of the Expressed Target Proteins by Western blotting (WB)

To confirm the expression of NP and M2e by the recombinant vaccinia viruses using Western blotting, CEF cells with 90% confluence grown in T25 flasks were infected with each strain of virus at MOI = 3. At 48 h post infection, the cell culture supernatants were removed and cells were harvested, total proteins were then extracted using Tissue Cell Total Protein Extraction Kit (Beijing Applygen Technologies Inc., Beijing, China). Furthermore, equal volume of 2×SDS-PAGE loading buffer was used to dissolve protein extracts, then they were boiled at 100 °C for 10 min, finally, the protein samples were analyzed with 4%–15% denaturing gradient gel electrophoresis and WB. The expression of β-Gal, NP and M2e was confirmed using mouse monoclonal antibody (MAb) against β-Galactosidase (40-1a) (sc-65670) (Santa Cruz, California, USA), mouse polyclonal antibody against influenza A virus NP (prepared in our laboratory), and mouse MAb against influenza A virus M2 (14C2) (ab5416) (abcam, Cambridge, UK). The signals were then scanned using Infrared Fluorescence-conjugated goat anti-mouse IgG (IRDye® 800CW) (ab216772) (abcam) and Odyssey Infrared Fluorescence Scanning Imaging System.

Identification of the Expressed Target Proteins by flow cytometry (FCM)

To confirm the expression of NP and M2e by the recombinant vaccinia viruses using FCM, A549 cells with 80% confluence in 6-well cell culture plate were infected with each strain of virus at MOI = 3. At 48 h post infection, cell culture supernatants were removed and cells were isolated by trypsin digestion method, and fixed with 4% paraformaldehyde, nonspecific sites on the cells were blocked up with 4% goat serum in Perm/Wash™ buffer (BD Biosciences, New Jersey, USA), furthermore, the expression of β-Gal, NP, and M2e were identified by chicken polyclonal antibody against β-Gal (ab9361) (abcam) and Alexa Fluor® 647-conjugated goat anti-chiken IgY H&L (abcam) (ab150171), mouse polyclonal antibody against influenza A virus NP (prepared in our laboratory) and Alexa Fluor 488-conjugated goat anti-mouse IgG (ab150171) (abcam), mouse MAb against influenza A virus M2

(14C2) (ab5416) (abcam) and Alexa Fluor® 488-conjugated goat anti-mouse IgG (ab150171) (abcam), respectively. The target cells were analyzed by FCM with dual-fluorescent detection method of Alexa Fluor® 647 and Alexa Fluor® 488, dual fluorescence positive cells were gated firstly, then the expression of NP and M2e in gated cells were analyzed.

Table S1 Expression of NP and M2e in recombinant virus infected cells

Antigen	Recombinant vaccinia virus	Average fluorescence reading	Ratio of fluorescence*
NP	RVJ1175	15919	1.0
	RVJ-NPM2e	98740	6.2
	RVJ-M2eNP	118668	7.4
	RVJ-4M2eNP	111371	7.0
	RVJ-4M2eNPs	23743	1.5
M2e	RVJ1175	8216	1.0
	RVJ-NPM2e	38518	4.7
	RVJ-M2eNP	46294	5.6
	RVJ-4M2eNP	75358	9.2
	RVJ-4M2eNPs	42018	5.1

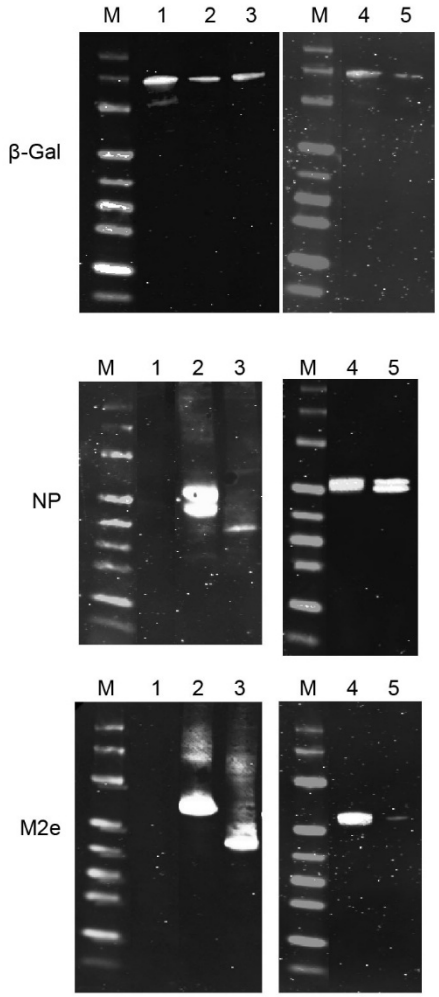

Fig. S1 The expression of target proteins by recombinant vaccinia virus in CEF were confirmed by Western blot analysis. CEF cells were infected by RVJ1175 (viral vector control), RVJ-NPM2e, RVJ-M2eNP, RVJ-4M2eNP, and RVJ-4M2eNPs. At 48 h post infection, the infected cells were harvested and labeled by mouse monoclonal or polyclonal antibodies to detect β-Gal (upper panel), influenza NP (middle panel), and M2e (lower panel), followed by incubation with infrared fluorescence-conjugated goat anti-mouse IgG (IRDye® 800CW). The signals were then scanned and analyzed using Odyssey Infrared Fluorescence Scanning Imaging System. M，protein molecular weight marker, the bands in the descending order of molecular weight are as follows: 170, 130, 100, 55, 40, 35, 25, 15, 10; lane 1, RVJ1175; lane 2, RVJ-4M2eNP; lane 3, RVJ-4M2eNPs; lane 4, RVJ-NPM2e; lane 5, RVJ-M2eNP.

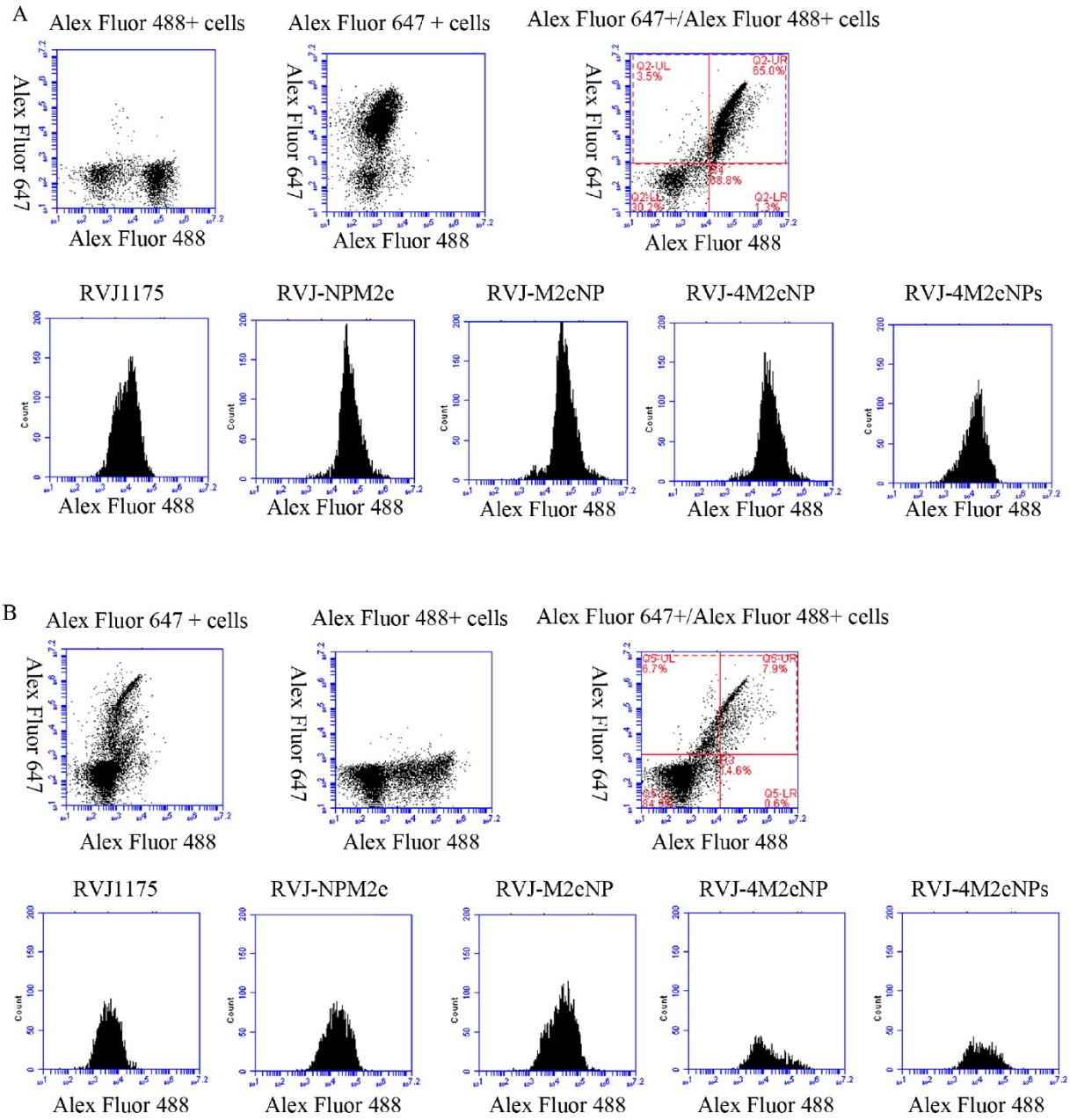

Fig. S2 The expression of target proteins by recombinant vaccinia virus in CEF were confirmed by FCM. A549 cells were infected by RVJ1175 (viral vector control), RVJ-NPM2e, RVJ-M2eNP, RVJ-4M2eNP, and RVJ-4M2eNPs. At 48 h post infection, the infected cells were harvested and labeled by chicken polyclonal Ab, mouse polyclonal Ab or MAb to detect β-Gal, influenza NP, and M2e, followed by incubation with matched Alexa Fluor® 647-conjugated goat anti-chiken IgY H&L or Alexa Fluor 488-conjugated goat anti-mouse IgG. The target cells were analyzed by FCM with dual-fluorescent detection method of Alexa Fluor® 647 and Alexa Fluor® 488, dual-fluorescent positive cells were gated firstly, and then the expression of NP (A) and M2e (B) in gated cells were analyzed.