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Running title: Foreign gene transduction into animal cells using BmNPV

# Transduction of a *Neospora caninum* antigen gene into mammalian cells using a modified *Bombyx mori* nucleopolyhedrovirus for antibody production

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**ABSTRACT**

***Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) can easily enter and transduce foreign genes into mammalian cells, but these functions are difficult for *Bombyx mori* nucleopolyhedrovirus (BmNPV). In this study, we investigated the induction of antibody production in mice immunized with an engineered BmNPV. The GP64 of BmNPV (BmGP64) was replaced with the GP64 of AcMNPV (AcGP64); this construct, designated BmNPVΔbgp/AcGP64, displays AcGP64 on the surface of BmNPV. The *Neospora caninum* antigen (NcSRS2) expression cassette, consisting of the cytomegalovirus immediate-early promoter and *NcSRS2* from *Neospora caninum*, was inserted into BmNPVΔbgp/AcGP64; this construct was designated BmNPVΔbgp/AcGP64/SRS2. For comparison, AcMNPV/SRS2, which contains the same NcSRS2 expression cassette as for BmNPVΔbgp/AcGP64, was also constructed. NcSRS2 was expressed in HEK293T cells when the engineered BmNPVs were transduced at a multiplicity of infection of 150. BmNPVΔbgp/AcGP64/SRS2 induced the production of NcSRS2-specific antibodies in mice, whereas AcMNPV/SRS2 and the control BmNPV did not. These results suggest that BmNPV prepared from silkworm hemolymph induces the production of antigen-specific antibodies in immunized mice and can be used for antibody production and vaccine development.**

[*Keywords:* AcMNPV; BmNPV; Transduction; Silkworm; Antibody production]

## INTRODUCTION

Baculovirus has been widely used for the production of recombinant proteins in insects and insect cells. Several types of baculovirus expression system that easily and rapidly express recombinant proteins are currently available (1−3). These baculovirus expression systems are useful for expressing and analyzing recombinant proteins, especially eukaryotic proteins. For example, *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) and *Bombyx mori* nucleopolyhedrovirus (BmNPV) have both been widely used as baculovirus expression systems.

It was previously reported that AcMNPV can transduce foreign genes into mammalian cells without any specific infection process (4,5). Foreign genes delivered into mammalian cells by AcMNPV are expressed if the genes are inserted under the control of a mammalian promoter; such a baculovirus is called a "BacMam." This BacMam, which contains antigen expression cassettes for mammalian cells, can be used as a carrier of DNA vaccines against infectious viruses and parasites (6). Baculovirus particles can also elicit an innate immunity in animals because they possess adjuvant activity, and their genomic DNA can be taken up by antigen-presenting cells (7−9). Therefore, these baculoviruses do not require any adjuvant for immunization into animals. Moreover, AcMNPVs containing an antigen expression cassette and displaying recombinant antigen have been developed as dual-function vaccines, serving as both subunit and DNA vaccines (10,11).

Few papers have reported the use of BmNPV for gene transduction into mammalian cells. Imai et al. reported the optimization of gene transduction into mammalian cells by BmNPV using phosphate-buffered saline (PBS) and sodium butyrate (12). We improved

the gene transduction efficiency by displaying GP64 of AcMNPV (AcGP64) on the surface of BmNPV (13). Optimizing the culture conditions and replacing an envelope protein on the surface of BmNPV enable this virus to transduce foreign genes into mammalian cells, although it is more difficult for BmNPV than it is for AcMNPV. However, BmNPV is better suited for the large-scale preparation of baculovirus particles than AcMNPV because BmNPV can be prepared from silkworm hemolymph, which contains high-titer BmNPV particles, without the need for specialized equipment (14).

In this study, we investigated the production of antibodies against *Neospora caninum* antigen (NcSRS2) using AcGP64-displaying BmNPVs containing the gene encoding NcSRS2 under the control of the human cytomegalovirus (CMV) immediate-early promoter. This study supports the application of baculoviruses in the fields of vaccine development and gene delivery.

## MATERIALS AND METHODS

**Construction of recombinant baculovirus bacmids** The DNA fragment of the CMV promoter, multiple cloning site (MCS) and bovine growth hormone polyadenylation signal (BGH pA) were amplified by polymerase chain reaction (PCR) using specific primers (CMV-F and BGH pA-R, as described in Table 1) as a template for the pcDNA 3.1 vector (Life Technologies Japan, Tokyo, Japan). The polyhedrin promoter, the MCS and Simian virus 40 late polyadenylation (SV40 pA) signal in the pFastbac 1 vector (Life Technologies Japan) were replaced with the amplified CMV promoter-MCS-BGH pA fragment by In-fusion technology (TaKaRa Bio, Ohtsu, Japan).

The pFastbac 1 vector, which contains a deleted polyhedrin promoter-MCS-SV40 pA region, was amplified using pFB-F and pFB-R primers (Table 1). The constructed plasmid was designated pFastbac/CMV. Full-length *NcSRS2* was amplified by PCR using primers (NcSRS2-F and NcSRS2-R, as described in Table 1) as per a previously constructed vector (15). This *NcSRS2* was inserted into the *Not*I and *Eco*RI sites located downstream of the CMV promoter in pFastbac/CMV. The constructed vector was designated pFast/c-NcSRS2. This vector was transformed into *Escherichia coli* DH10Bac cells (Life Technologies Japan), and the recombinant AcMNPV bacmid was extracted from a white transformant. This AcMNPV was designated AcMNPV/SRS2.

To construct a recombinant BmNPV that transduces foreign genes into mammalian cells, *NcSRS2* and *AcGP64* were inserted downstream of the CMV and p10 promoters, respectively, in pFastbacdual/p10/CMV (13). *NcSRS2* was amplified by PCR using NcSRS2-F and NcSRS2-R primers (Table 1), and *AcGP64* was amplified by PCR using AcGP64-F and AcGP64-R primers. The resulting plasmid was transformed into *E. coli* BmDH10Bac/ΔBmGP64 (13), and the recombinant BmNPV bacmid was extracted using the same procedure described for AcMNPV/SRS2. This recombinant BmNPV displays AcGP64 and contains the NcSRS2 expression cassette for expression of *NcSRS2* from *N. caninum*, but it lacks BmGP64. This bacmid was designated BmNPVΔbgp/AcGP64/SRS2. Control AcMNPV and BmNPV bacmids, which do not contain the NcSRS2 expression cassette, were extracted from *E. coli* DH10Bac cells and *E. coli* BmDH10Bac cells (16), respectively.

Full-length *NcSRS2* was amplified using primers F-NcSRS2-F and F-NcSRS2-R. The amplified gene was inserted into pcDNA3.1 (+) (Life Technologies Japan), and pcDNA/SRS2 was constructed as a DNA vaccine.

**Preparation and titer determination of recombinant baculoviruses** The recombinant AcMNPV bacmid was transfected into Sf-9 cells using Cellfectin II (Life Technologies Japan) according to the manual provided with the Bac-to-Bac expression system (Life Technologies Japan). Sf-9 cells were maintained in Sf-900II Serum-Free Medium (Life Technologies Japan) supplemented with 10% fetal bovine serum (Life Technologies Japan) and an antibiotic-antimycotic (Life Technologies Japan). Virus amplification was also performed according to the manual provided with the Bac-to-Bac expression system. The culture supernatant was centrifuged at 10000 × g to remove cells and cell debris and then at 96000 × g to pellet the recombinant AcMNPV particles. The pelleted particles were resuspended in PBS (pH 7.4). The suspension was used as a recombinant AcMNPV solution after titer determination.

The recombinant BmNPV bacmid was injected into silkworm larvae using DMRIE-C Reagent (Life Technologies Japan). Hemolymph was collected after baculovirus infection and centrifuged on a 25% sucrose layer at 96000 × g. The pellet was resuspended in PBS, and a 25−60% sucrose density gradient centrifugation was performed at 96000 × g. Recombinant BmNPV particles were collected and dialyzed against PBS. The suspension was used as a recombinant BmNPV solution following titer determination.

Titer determination of recombinant AcMNPV was performed according to a previous report (17) using primers Ac-ie1 F and Ac-ie1 R (Table 1). Titer determination for BmNPV was also performed according to a previous report (14) using the primers Bm-ie1 F and Bm-ie1 R (Table 1).

**Baculoviral transduction into mammalian cells and immunofluorescence microscopy** HEK293T cells were maintained at 27°C in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific K.K., Tokyo, Japan) supplemented with 10% horse serum (Thermo Fisher Scientific K.K.) and 1% non-essential amino acids (Thermo Fisher Scientific K.K.) in a 5% $CO_2$ environment. For the transduction of recombinant AcMNPV, $2 \times 10^5$ HEK293T cells were transduced with each recombinant AcMNPV at an M.O.I. of 150, followed by incubation for 72 h. In the case of pcDNA/SRS2, 2 μg of pcDNA/SRS2 was transfected into $2 \times 10^5$ HEK293T cells using Lipofectamine 2000 (Life Technologies Japan). Trypsinized cells were placed onto an aminosilane-coated glass slide. After cell fixation with 4% paraformamide for 20 min and treatment with 50 mM ammonium chloride, cells were blocked with 8% bovine serum albumin (BSA) for 2 h and washed with PBS. Cells were treated with mouse anti-NcSRS2 serum (18) diluted with 2% BSA in PBS (1:200) and incubated for 1 h. After washing 3 times with PBS, cells were incubated with Alexa 594-conjugated anti-mouse IgG (Jackson ImmunoResearch Laboratory, West Grove, PA, USA) diluted with 2% BSA in PBS (1:200). Cells were washed 3 times with PBS. 4',6-Diamidino-2-phenylindole Dihydrochloride n-Hydrate (DAPI, Wako Pure Chemical, Osaka, Japan) was used at 1 μg/ml to stain nuclei in cells. Blue and red fluorescence signals were observed by confocal laser scanning microscopy (LSM700, Carl Zeiss Japan, Tokyo, Japan).

**Immunization of recombinant baculoviruses into mice** Female BALB/c mice (6 weeks of age) were purchased from Japan SLC, Inc. (Shizuoka, Japan) and housed under conventional day/night conditions. At the age of 7 weeks, mice were randomly

distributed into groups of 5 mice each. Each mouse was inoculated with each baculovirus. Mice in Group 3 received PBS alone (infection control group). One hundred microliters of recombinant AcMNPV particles ($1 \times 10^8$ pfu) or 100 μg of pcDNA/SRS2 was injected into the mice intramuscularly. Injection was performed three times every two weeks for immunization (n=5). Whole blood was collected two weeks after the last injection, and serum was prepared by centrifugation at 1,000 × g.

**Enzyme-linked immunosorbent assay (ELISA)** To determine the titer of antibodies to each antigen in mouse serum, 100 ng of NcSRS2 purified from silkworms (18) was immobilized onto one well of a 96-well plate. The supernatant was removed, and 2% skimmed milk in TBST was added to each well and incubated for 1 h. The skimmed milk solution was removed, and diluted serum was added to each well. After incubation at room temperature for 1 h, each well was washed 3 times with TBST; anti-mouse IgG antibody-horseradish peroxidase (HRP, GE Healthcare Japan) diluted 5000-fold with TBST was then added to each well and incubated at room temperature for 1 h. Each well was washed with TBST, followed by reaction with HRP. One hundred microliters of substrate (0.1 mg/ml 3,3′,5,5′-tetramethylbenzidine in 100 mM sodium acetate, pH 6.0, with 0.2% (v/v) of 30% hydrogen peroxide) was added to each well and left at room temperature for color development. The reaction was stopped by the addition of 50 μl 1N $H_2SO_4$ solution, and the resulting color was measured at an optical density (OD) of 450 nm. The antibody titer was determined as the highest fold dilution that reached the same value as the negative control (PBS-injected mouse serum) by linear regression analysis with coefficient determination ($R^2$) > 0.9.

## RESULTS

**Expression of NcSRS2 in mammalian cells with recombinant AcMNPVs and BmNPVs** Baculoviruses can be used as DNA vaccines against infectious viruses and parasites by expressing antigens in mammalian cells. To achieve this goal, the expression of antigens should be controlled by a mammalian promoter. In this study, the CMV promoter was used to express NcSRS2 in mammalian cells. The NcSRS2 expression cassette was constructed using the CMV promoter and inserted into AcMNPV and BmNPVΔbgp bacmids (Fig. 1). Deletion of *BmGP64* in BmNPV prevented the amplification of BmNPV in host cells (13). To rescue this amplification and help BmNPV transduce a foreign gene into mammalian cells, *AcGP64* was inserted under the control of the p10 promoter together with *NcSRS2* under the control of the CMV promoter. Using these recombinant baculovirus bacmids, AcMNPV/SRS2 and BmNPVΔbgp/AcGP64/SRS2 particles were prepared in Sf-9 cells and silkworm larvae, respectively.

When AcMNPV/SRS2 or BmNPVΔbgp/AcGP64/SRS2 particles were transduced into HEK293T cells at an M.O.I. of 150, NcSRS2 was expressed (Fig. 2). pcDNA/SRS was used as a positive control to express NcSRS2 in mammalian cells. Similarly, NsSRS2 was also expressed in HEK293T cells following transfection with pcDNA/SRS2, which is a pcDNA vector containing the NcSRS2 gene under control of the CMV promoter,. The expression level of NcSRS2 using both recombinant AcMNPV and BmNPV was lower than that using pcDNA/SRS2. Wild-type BmNPV particles containing the same NcSRS2 expression cassette as AcMNPV/c-SRS2 did not induce the expression of NcSRS2 in HEK293T cells at a M.O.I. of 500 (data not shown). These

results indicate that AcMNPV/SRS and BmNPVΔbgp/AcGP64/SRS2 particles facilitate the expression of NcSRS2 under control of the CMV promoter in mammalian cells.

**Immunization of recombinant baculoviruses in mice** Immunization of BmNPV or BmNPVΔbgp/AcGP64/SRS2 in mice was performed, and NcSRS2-specific antibody production was investigated. The antibody level against recombinant NcSRS2 purified from silkworm hemolymph was measured in mice immunized with BmNPV or BmNPVΔbgp/AcGP64/SRS2. The antibody level in mice immunized with BmNPVΔbgp/AcGP64/SRS2 was higher than that in mice immunized with BmNPV (Fig. 3A). However, some antibodies in mice immunized with BmNPV reacted with recombinant NcSRS2 purified from silkworm hemolymph in the ELISA used to measure antibody levels. This outcome indicated that some antibodies against the proteins from silkworm hemolymph were induced in mice by the immunization of BmNPVΔbgp/AcGP64/SRS2 particles, and these antibodies may react to the proteins from silkworm hemolymph in purified NcSRS2 in the ELISA. Therefore, we carried out indirect ELISA after the serum of mice immunized with BmNPVΔbgp/AcGP64/SRS2 or control BmNPV particles was mixed with silkworm hemolymph to capture antibodies to the proteins from silkworm hemolymph (Fig. 3B). The absorbance in the serum of mice immunized with control BmNPV particles was reduced compared to that in the serum of mice immunized with PBS when antibodies to proteins in silkworm hemolymph were captured. However, the antibodies to NcSRS2 were detected in the serum of mice immunized with BmNPVΔbgp/AcGP64/SRS2 particles after antibodies to proteins in silkworm hemolymph were captured. These results clearly show that

BmNPVΔbgp/AcGP64/c-SRS2 particles induced the expression of NcSRS2 and the production of antibodies against NcSRS2 in mice.

Particles from each baculovirus (AcMNPV/SRS2 and BmNPVΔbgp/AcGP64/c-SRS2) and pcDNA/SRS2 were immunized into mice 3 times every 2 weeks. The sera from immunized mice were collected, and the production of antibodies against NcSRS2 was confirmed using an indirect ELISA. Antibodies against NcSRS2 were detected in the serum from mice immunized with BmNPVΔbgp/AcGP64/SRS2 or pcDNA/SRS2. AcMNPV/SRS2 did not induce the production of NcSRS2-specific antibodies in mice (Fig. 4). In a previous report, pcDNA harboring NcSRS2 induced NcSRS2-specific antibody production in mice (19). It was also previously reported that AcMNPV facilitated antigen-specific antibody production in mice as a DNA vaccine (10,20,21). However, in the current study, NcSRS2-specific antibody production was not induced in mice immunized with AcMNPV/SRS2. The expression level of NcSRS2 in HEK293T cells transduced with AcMNPV/SRS2 was low compared with that in HEK293T cells transduced with AcMNPV/EGFP (13) (data not shown), which may be one reason why AcMNPV did not induce NcSRS2-specific antibody production. By contrast, BmNPVΔbgp/AcGP64/SRS2 and pcDNA/SRS2 induced NcSRS2-specific antibody production.

## DISCUSSION

We previously described gene transduction into mammalian cells using AcGP64-displaying BmNPV (BmNPVΔbgp/AcGP64) (13). AcGP64 displayed on the surface of this recombinant BmNPV facilitates the expression of foreign genes in mammalian cells. We evaluated this AcGP64-displaying BmNPV as a DNA vaccine

against *N. caninum*. The BmNPVΔbgp/AcGP64/SRS2 prepared in this study induced the production of NcSRS2-specific antibodies in mice, suggesting that this recombinant BmNPV, as well as pcDNA3.1, can be used as a DNA vaccine vector. Apart from this AcGP64-displaying BmNPV, it was reported that BmNPV can mediate gene expression in mammalian cells by optimizing its transduction conditions (12). In this case, GFP expression was observed in HEK293T cells with the addition of PBS during BmNPV transduction. This method (i.e., the addition of PBS) can be applied to the in vitro expression of foreign genes in cultured cells. However, in vivo, the application of this method is difficult compared to the AcGP64-displaying BmNPV method used in this study.

The NcSRS2 expression level in HEK293T cells using AcMNPV/SRS2 or BmNPVΔbgp/AcGP64/SRS2 was lower than that using pcDNA/SRS2, suggesting that the gene transduction efficiency of AcMNPV/SRS2 and BmNPVΔbgp/AcGP64/SRS2 was comparatively low. In the case of BmNPVΔbgp/AcGP64/SRS2, its transduction efficiency can be improved by augmenting the level of AcGP64 expression on the surface of recombinant BmNPV; the AcGP64 expression level on the surface of recombinant BmNPV was lower than that on the surface of wild-type AcMNPV [13]. In this study, BmNPVΔbgp/AcGP64/SRS2, as well as pcDNA/SRS2, induced the production of antibodies against NcSRS2 in mice even though the NcSRS2 expression level in HEK293T cells transduced by BmNPVΔbgp/AcGP64/SRS2 was low. BmNPVΔbgp/AcGP64/SRS2 and control BmNPV particles were prepared from silkworm hemolymph via sucrose density gradient centrifugation. Compared to AcMNPV/SRS2 prepared from cell culture supernatant, a small amount of protein from

silkworm hemolymph was contained in the recombinant BmNPV particles. The induction of antibody production may have been partly caused by the presence of proteins from silkworm hemolymph in the BmNPVΔbgp/AcGP64/SRS2 sample that were not present in the AcMNPV/SRS2 sample. In fact, the hemocyanin of *Helix pomatia*, which is an edible snail (escargot), was recently investigated as a bio-adjuvant to enhance cellular and humoral immune responses in mice (22). Silkworm hemolymph contains a protein, pro-phenol oxidase (23), with an amino acid sequence similar to that of hemocyanin. Another advantage of using BmNPV as a DNA vaccine carrier is that it can be easily prepared in silkworm larvae.

Previous studies have reported that AcMNPV can induce the production of antigen-specific antibodies in immunized mice but that the production of NcSRS2-specific antibodies was not observed. We determined the titer of recombinant AcMNPV by quantitative real-time PCR. Our results suggest that the titer of recombinant AcMNPV in immunized mice was overestimated compared with that determined by the plaque assay method (24). Additionally, the expression level of NcSRS2 in HEK293T cells was low when using pcDNA/SRS2, AcMNPV/SRS2 and BmNPVΔbgp/AcGP64/SRS2 (Fig. 2) relative to the GFP expression observed in HEK293T cells using BmNPVΔbgp/AcGP64/EGFP (13), suggesting that NcSRS2 is not expressed efficiently in mammalian cells.

In this study, we constructed AcMNPV/SRS2 and BmNPVΔbgp/AcGP64/SRS2. NcSRS2 was expressed in HEK293T cells using both types of baculovirus particles. However, only BmNPVΔbgp/AcGP64/SRS2 particles induced the production of NcSRS2-specific antibodies when these baculovirus particles were immunized into mice. These results indicate that BmNPV from silkworm hemolymph is superior to

AcMNPV as a baculoviral DNA vaccine. The proteins of silkworm hemolymph may play a role in the induction of antibody production in mice because BmNPVΔbgp/AcGP64/c-SRS2 particles were not completely purified from silkworm hemolymph. It is necessary to investigate which proteins in silkworm hemolymph contribute to inducing the production of the antigen-specific antibodies in mice. The production of BmNPV particles in silkworm larvae can also be used for the development of baculoviral DNA vaccines.

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**Table 1.** Primers used in this study

| Name | 5’ to 3’ |
|---|---|
| pFB-F | TGATCACTGCTTGAGCCTA |
| pFB-R | CTATTAATATTCCGGAGTA |
| CMV-F | CCGGAATATTAATAGGTTGACATTGATTA |
| BGH pA-R | CTCAAGCAGTGATCACCATAGAGCCCAC |
| NcSRS2-F | ATGAATTCATCATGGCGACGCATGCTTGTG |
| NcSRS2-R | GCTCTAGACTAGTACGCAAAGATTGCC |
| AcGP64-F | AGGCCCCGGGATGCTACTAGTAAATCAGTCAG |
| AcGP64-R | GGCGCCCGGGTTAATATTGTCTATTACGGTTTC |
| F-NcSRS2-F | ATGAATTCATCATGGCGACGCATGCTTGTGT |
| F-NcSRS2-R | TAGCGGCCGCCTAGTACGCAAAGATTGCC |
| Ac-ie1 F | CCCGTAACGGACCTCGTACTT |
| Ac-ie1 R | TTATCGAGATTTATTTGCATACAACAAG |
| Bm-ie1 F | CCCGTAACGGACCTTGTGCTT |
| Bm-ie1 R | TTATCGAGATTTATTTACATACAACAAG |

## FIGURE LEGENDS

FIG. 1. Recombinant baculoviruses constructed in this study. The BmNPVΔbgp bacmid, which lacks the *BmGP64* gene, was constructed previously (13). To supplement GP64 on the surface of BmNPV particles, the *AcGP64* gene from AcMNPV was inserted under the control of the p10 promoter.

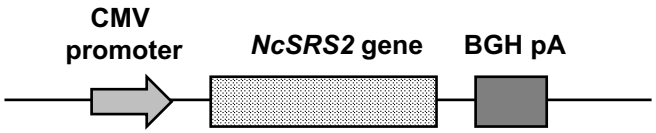
FIG. 2. Immunofluorescence microscopy of HEK293T cells. (A) HEK293T cells transfected with pcDNA, AcMNPV or BmNPVΔbgp/AcGP64. (B) NcSRS2-expressing HEK293T cells transfected with pcDNA/SAR2, AcMNPV/SRS2 or BmNPVΔbgp/AcGP64/SRS2. In the case of pcDNA/SRS2, 2 μg of the vector was transfected into HEK293T cells. In the case of baculoviruses, each baculovirus was transduced into HEK293T cells at an M.O.I. of 150. Cells were stained with mouse anti-NcSRS2 antibody and Alexa 594-conjugated anti-mouse IgG. DAPI was used to stain nuclei in these cells.

FIG. 3. Detection of antibodies against recombinant NcSRS2 in serum from mice immunized with PBS, BmNPV, or BmNPVΔbgp/AcGP64/SRS2 (A). Briefly, $1 \times 10^8$ pfu of each baculovirus was immunized into mice 3 times every 2 weeks. The serum was prepared, and the antibodies to NcSRS2 were detected by ELISA using NcSRS2 purified from silkworm hemolymph. Detection of antibodies to NcSRS2 in the serum of mice immunized with each baculovirus after mixing with silkworm hemolymph (B). The serum was mixed with silkworm hemolymph diluted 5- or 10-fold with PBS, and ELISA was used to detect NcSRS2-specific antibodies using NcSRS2 purified from silkworm hemolymph.

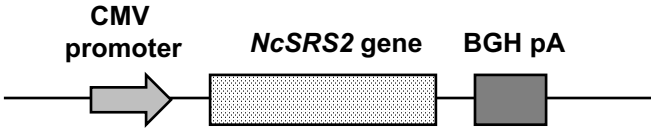
FIG. 4. Antibody titer measurement of recombinant NcSRS2 in mice immunized with pcDNA/SRS2, AcMNPV/SRS2, or BmNPVΔbgp/AcGP64/SRS2. In the case of

pcDNA/SRS2, 100 μg of the vector was immunized into mice three times every two weeks. Each baculovirus was immunized at $1 \times 10^8$ pfu in mice three times every two weeks. The serum was prepared, and the antibodies to NcSRS2 were detected by ELISA usin-+g NcSRS2 purified from silkworm hemolymph.

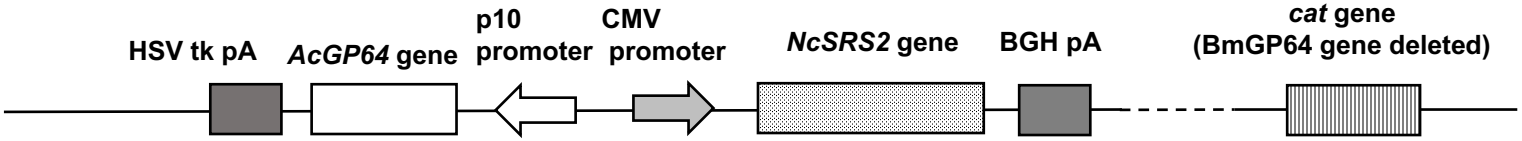
**pcDNA/SRS2**

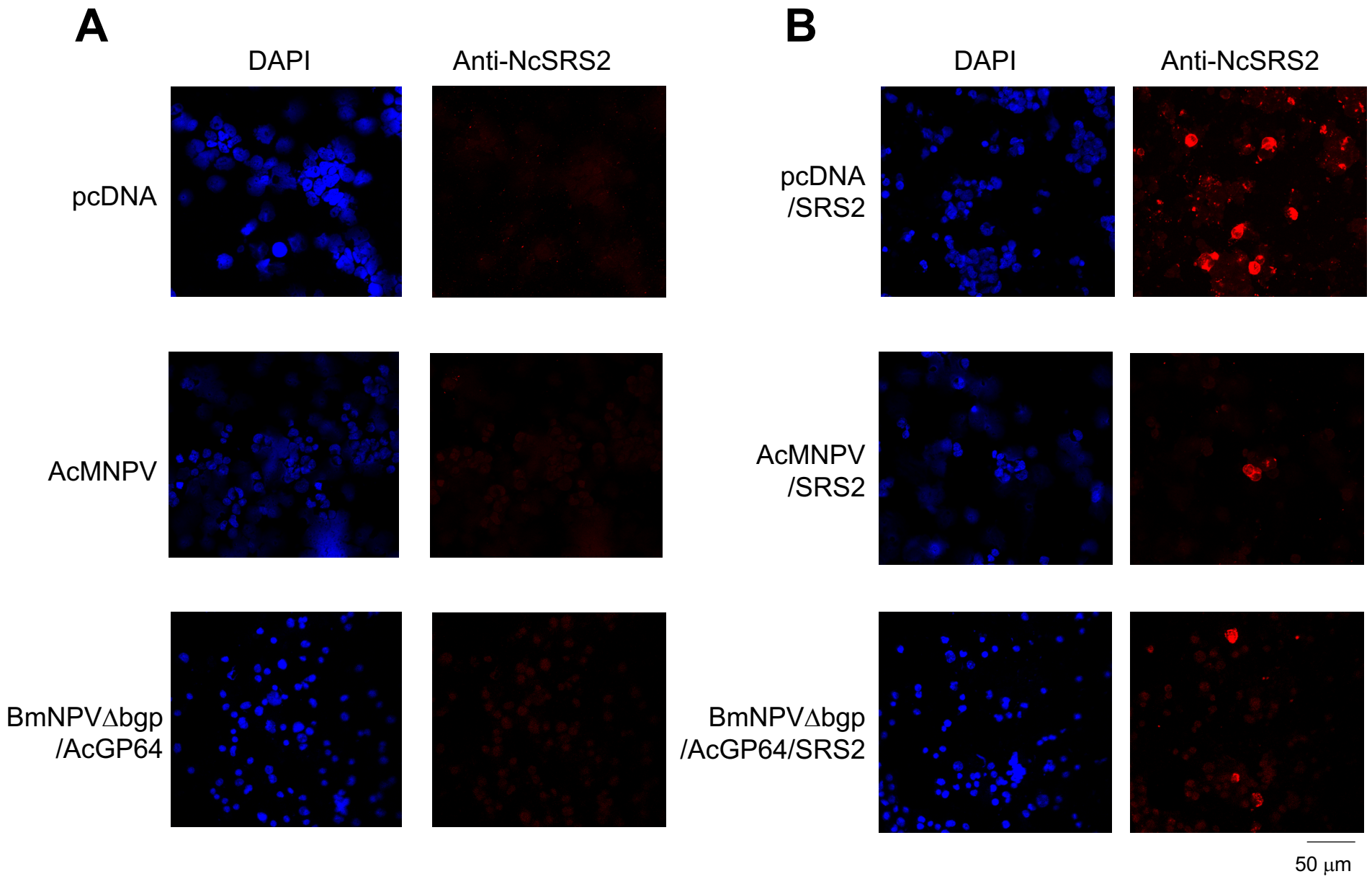


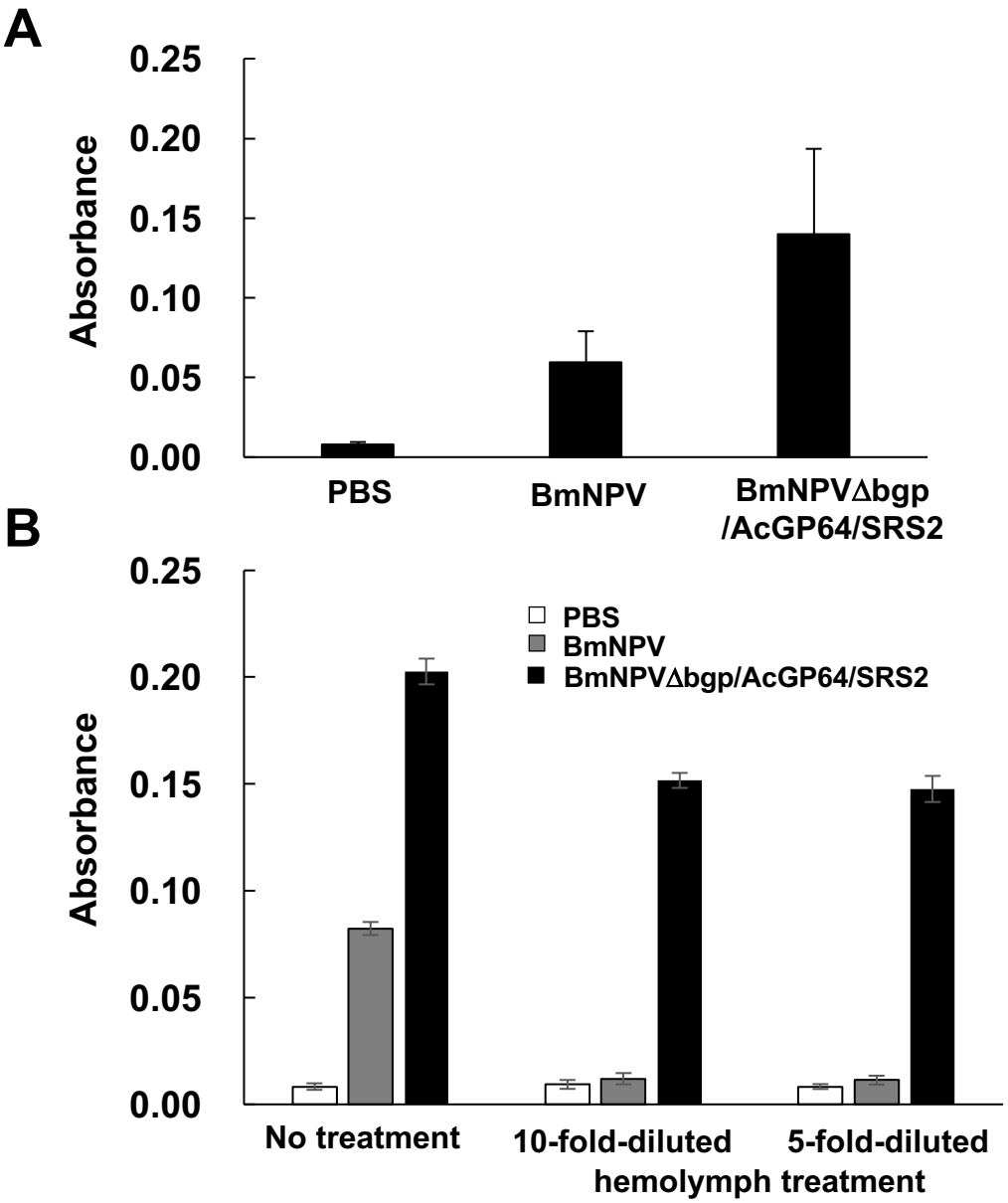

**AcMNPV/SRS2**

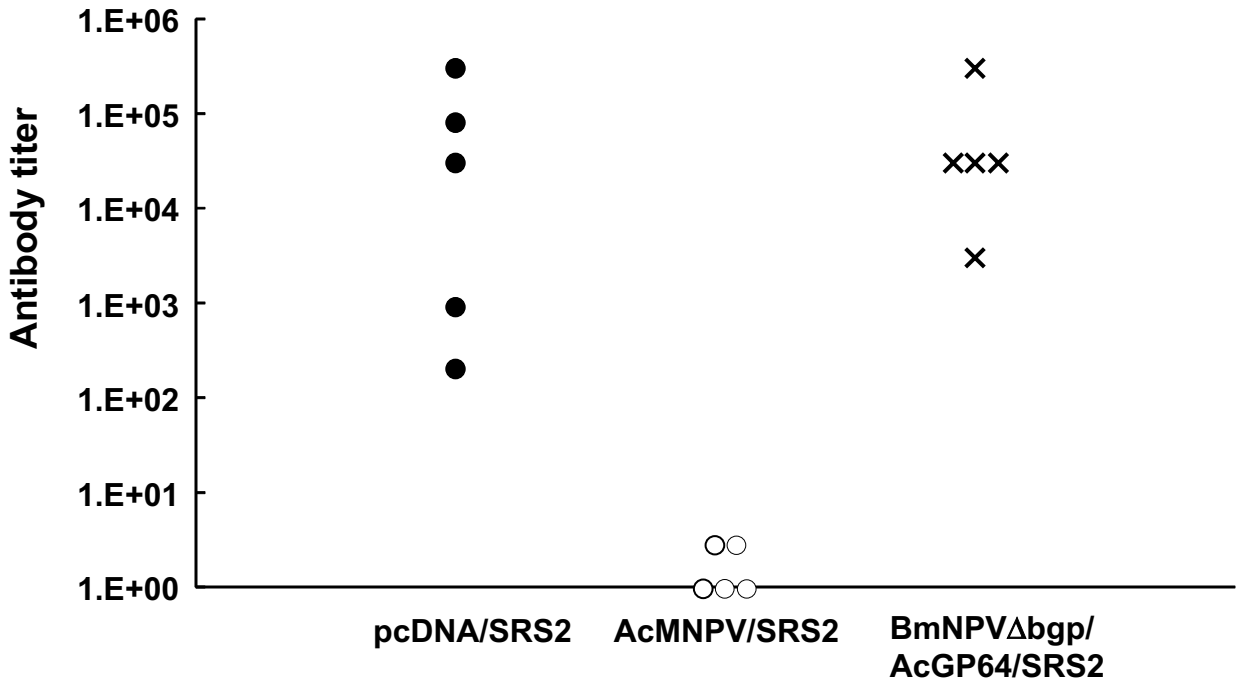



**BmNPVΔbgp/AcGP64/SRS2**




A
DAPI
Anti-NcSRS2
pcDNA
AcMNPV
BmNPVΔbgp
/AcGP64
B
DAPI
Anti-NcSRS2
pcDNA
/SRS2
AcMNPV
/SRS2
BmNPVΔbgp
/AcGP64/SRS2
50 μm


A
Absorbance
0.25
0.20
0.15
0.10
0.05
0.00
PBS
BmNPV
BmNPVΔbgp
/AcGP64/SRS2
B
Absorbance
0.25
0.20
0.15
0.10
0.05
0.00
PBS
BmNPV
BmNPVΔbgp/AcGP64/SRS2
No treatment
10-fold-diluted
5-fold-diluted
hemolymph treatment


Antibody titer
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
pcDNA/SRS2
AcMNPV/SRS2
BmNPVΔbgp/
AcGP64/SRS2