**Supplementary Information**

The method used to culture host cells (Sf9 cells) can affect the qualities of baculovirus budding particles expressing recombinant proteins

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**Supplementary Information 1: Construction of recombinant AcNPV**

The recombinant baculovirus (ADRB2-TagGFP2 BV) was constructed according to both conventional protocols and previous studies [Ref. 34 in the main text]. Briefly, the gene *ADRB2* (Missouri S&T cDNA Resource Center, Rolla, MO; Clone ID AR0B200000; GenBank Acc. No. NM\_000024.3) on pcDNA3.1+ was amplified by PCR (PrimeSTAR® HS DNA Polymerase, Takara Bio Inc., Otsu, Japan) with primers (forward agtgtggtgg aattcgatta tccaccatgg ggcaacc; reverse gcgaccggtg aacctccacc cagcagtgag tcatttgtac) including *Eco*R I and *Age* I sites. The plasmid DNA coding TagGFP2 (pTagGFP2-N, Evrogen) was digested with these restriction enzymes, subjected to dephosphorylation with alkaline phosphatase (calf intestine, Takara Bio), and purified using agarose gel electrophoresis. The linearized plasmid was ligated (DNA Ligation Kit Ver. 2.1, Takara Bio) with the above PCR fragment as an insert that was treated with the restriction enzymes in advance. The plasmid coding an ADRB2-TagGFP2 was prepared by transformation into competent cells (ECOSTM Competent E.coli DH5α, Nippon Gene Co., Ltd., Toyama, Japan). The fragment of ADRB2-TagGFP2 that was digested using *Eco*R I and *Not* I from the plasmid was ligated as an insert to the *Eco*R I/*Not* I-treated baculovirus transfer vector pVL1393 (BD Biosciences, San Diego, CA), which was subjected to transformation. Sf9 cells were co-transfected with the vector pVL1393 coding ADRB2-TagGFP2 and the linearized baculovirus genome DNA (BD BaculoGoldTM linearized Baculovirus DNA) according to the provided user’s guide, except that Cellfectin® II (Life Technologies) was adopted for transfection instead of the reagent provided. The desired recombinant AcNPV BVs were obtained in the culture supernatant after recombination inside host cells, and, as necessary, the BV titer was amplified.

**Supplementary Information 2: Estimation of MOI values**

An MOI value is deduced by dividing the number of viruses by the number of the cells to which the viruses are added. To estimate the MOI of each procedure (i) standing, (ii) shaking or (iii) standing/shaking, we counted the typical number of Sf9 cells in culture media that would be just subjected to the infection using a cell counter (Merck-Millipore ScepterTM, handheld automated cell counter, with a 40 μm sensor). The number of cells cultured in a 75 cm2 flask with 11.5 mL Sf-900TM III SFM (for (i) and (iii)) and a 250 mL plastic-baffled bottle with 110 mL of the SFM (for (ii)) was obtained to be 2.15 × 107 (1.87 × 106/mL × 11.5 mL) per flask and 1.38 × 108 (1.25 × 106/mL × 110 mL) per bottle, respectively: The numbers are summarized in the following table.

The titer (the number of virions per unit volume (1 mL)) of the seed ADRB2-TagGFP2 BV (6th passage) was estimated by direct observation on TagGFP2-expressing infected cells with an inverted fluorescence microscope (Nikon TE300) equipped with a 10× objective lens, a FITC-filter and a Hamamatsu ORCA®-ER CCD camera/AQUACOSMOSTM image acquisition system. A suspension (0.4 mL) of Sf9 cells that had been cultured to full confluence in ~5 mL Sf-900TM III SFM of a 25 cm2 flask was placed on to a well of a 6-well plastic plate (Sumitomo Bakelite Co., Ltd., MS-80060; the culture area, 9.2 cm2), 2 mL SFM was added thereto, and the plate was incubated at 27 °C for 24 hours. Then, the seed BV was 100-fold diluted with the SFM, and 0.5 mL of the dilution was added to the well from which the medium had been removed in advance. The plate was further incubated for 18 hours, 1.5 mL of fresh SFM was added thereto and incubated for more 30 hours, and the SFM was completely removed and the cells were subjected to the fluorescence microscopic observation 48 hours after the infection. Capturing seven images (the area of view is 0.87 mm × 0.66 mm (= 0.57 mm2)) of cells that covered the bottom surface of the well, we counted TagGFP2-expressing cells with the help of the image processing software ImageJ. The mean number is 156.7 cells per view, and so 2.5 × 105 infected cells per well. Considering the dilution factor (100) and the added volume containing the BV (0.5 mL of the dilution 1 mL), we could estimate the titer of the BV used here to be 5.0 × 107 (or 0.50 × 108) pfu per mL. Although the value seemed to be underestimated, we adopted it.

Therefore, we could roughly estimate the typical MOI values about the present study as shown in the following table. It is to be noted that the order of the value may be meaningful due to internal errors and fluctuation with bath-type experiments.

Table: MOI’s estimated from typical cell cultures with the three procedures

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Procedure | Number of Sf9 cells *A* | Number of ADRB2-TagGFP2 BVs *B* | | MOI *B/A* |
| (i) Standing | 1.51 × 108 a | | 0.50 × 108 | 0.33 |
| (ii) Shaking | 1.08 × 108 b | | 0.50 × 108 | 0.46 |
| (iii) Standing/shaking | 1.38 × 108 | | 0.50 × 108 | 0.36 |

a Ten flasks where Sf9 cells had been cultured to 70 % confluence were used.

b Five flasks where Sf9 cells had been cultured to 100 % confluence were used.

**Supplementary Information 3: Western blot (WB)**

BVs were analyzed by WB as follows. Three microliters of protein marker (Precision Plus ProteinTM Dual Color Standards, Bio-Rad) and 4 μL portions of the suspension from Fraction 4 were loaded on a polyacrylamide gel (8 cm × 9 cm) that consisted of stacking gel (3.89 % (w/v) acrylamide (Wako), 0.11 % (w/v) *N,N'*-methylene-bisacrylamide (BIS; Wako), 0.1 % (w/v) SDS (Wako), 125 mM Tris-HCl (pH 6.8) and 0.01 % (w/v) ammonium persulfate (APS; Bio-Rad), 0.001 % (w/v) *N,N,N',N'*-tetramethylethylenediamine (TEMED; Bio-Rad)) and running gel (12 % acrylamide, 0.41 % BIS, 0.1 % SDS, 375 mM Tris-HCl (pH 8.8), and 0.01 % APS, 0.001% TEMED), and electrophoresed at 30 mA per gel for 50 min using a PAGE apparatus (Dual Mini Slab; ATTO Corporation, Tokyo, Japan) with running buffer (0.1 % SDS, 25 mM Tris (pH 8.4), 52 mM glycine (Wako)). A piece of polyvinylidene fluoride membrane (Bio-Rad Immun-Blot PVDF) and the gel with some pieces of filter paper were immersed for 15 min at 4 °C in methanol and transfer buffer (48 mM Tris, 39 mM glycine, 20 % (v/v) methanol (Wako)), respectively. Proteins were transferred from the gel to the membrane using a blotter (Bio-Rad Trans-Blot® TurboTM Transfer System). The membrane was set on an immunoblot apparatus (Merck-Millipore SNAP i.d.® 2.0 Protein Detection System) according to the provided user’s guide. The membrane was treated with 30 mL of blocking solution (0.5 % (w/v) skim milk (Immunoblot Blocking Reagent, Merck-Millipore), 0.1 % (v/v) Tween 20 (Sigma-Aldrich) in PBS (pH 7.4)), a primary antibody solution (anti-Tag(CGY) FP antibody (Evrogen) in 3 mL of 0.1 % Tween 20/Can Get Signal® Solution 1 (Toyobo Co., Ltd., Osaka, Japan)) and a secondary antibody solution (anti-rabbit IgG, horseradish peroxidase (HRP)-conjugate (Santa Cruz Biotechnology) in 3 mL of 0.1 % Tween 20/Can Get Signal® Solution 2), in this order. The blotting reaction was carried out using an enhanced chemiluminescent (ECL) substrate (Thermo Scientific SuperSignal® West Femto Maximum Sensitivity Substrate) according to the provided user’s guide and the signal was imaged with a CMOS camera-equipped device (ImageCapture G3, Liponics, Inc., Tokyo, Japan).

**Supplementary Information 4: Estimation of the relative amounts of intact BVs**

The amounts of intact BVs contained in the lanes of the Fractions 4 with the three procedures were estimated from the extent to which the bands were visualized by CBB staining. Using the image processing software ImageJ, the color mode of the SDS-PAGE image (Fig. 3(A)) was converted to the gray scale (8-bit) and then inverted. Within the band of GP64 or VP39 of the three lanes or the background, a certain rectangular region of interest was set, and the mean value of the intensity was arbitrarily obtained. A difference between the value and the background intensity was compared to each other.