1. Materials and methods

Cell culture and virus production: *Spodoptera frugiperda* 9 (Sf9) cells were cultured with Grace's Insect Medium (Invitrogen, USA) supplemented with 10%(v/v) foetal bovine serum (FBS, Gibco) at 28 °C. Recombinant baculovirus were rescued through the Bac-to-Bac system (Invitrogen). Briefly, the BirAAP sequence on pFastBacDual-BirAAPGP64-VP39GFP[1] was replaced with SpyTag through molecular cloning. First, two PCR fragments generated by using primers A and B, which contain the SpyTag coding sequence and an additional 15-base-pair homology sequence for Sequence- and Ligase-Independent Cloning (SLIC). Then, the fragments were subjected to SLIC using the Clonexpress II one step clone kit (Vazyme Biotech, China) to generate the pFastBacDual-GP64spy-VP39GFP plasmid. The plasmid was transformed into DH10Bac competent cells to obtain the recombinant bacmid, bAc-GP64spy-VP39GFP. The recombinant virus AcVGspy was rescued by directly transfected bAc-GP64spy-VP39GFP Sf9 cells. Previously constructed Ac-VP39GFP[1] was used as a control and designated AcVG in this study.

Protein expression and purification: Cys-SpyCatcher protein expression and purification were performed as reported by Zakeri et al[2]. Briefly, the SpyCatcher gene was amplified from pET28a SpyCatcher-SnoopCatcher (addgene #72324) and inserted into pET28a with a cysteine at its N terminus. The generated plasmid pET28a-CysSpyCatcher was transformed into BL21(DE3). For protein expression, transformed bacteria were grown to OD600~0.8 at 37 °C and chilled on ice. IPTG was added with a final concentration of 1 mM to induce protein expression at 30 °C for 6 hours. Proteins were purified using an AKTA protein purification system through a HisTrap column. The buffer was exchanged with PBS (0.1 M, pH 7.4) through ion exchange chromatography.

QD605-SpyCatcher preparation: First, 100 μL of QD605-NH2 (8.3 μM, Invitrogen) was washed twice in a 100 kDa cutoff ultrafilter tube (Millipore) and resuspended in 100 μL of PBS (0.1 M, pH 7.4). Next, 1.5 μL of Mal-PEG4-NHS (150 μM in DMSO, Santa Cruz) was added to allow reaction at room temperature (RT) with rotation for 1 hour. Abundant Mal-PEG4-NHS was removed using a NAP-5 desalting column. For protein conjugation, 100 nmol of purified His-Cys-SpyCatcher was mixed with 5 mM TCEP and kept at RT for 5 minutes. The modified QDs, QD605-Mal complex was then mixed with His-Cys-SpyCatcher protein. The reaction was carried out 4 °C overnight. Unreacted His-CysSpyCatcher proteins were removed through a 100 kDa cutoff ultrafilter tube (Millipore) 5 times. The final product was resuspended in TBS buffer and storage at 4 °C until use.

Labelling of GP64spy proteins or AcVGspy viruses: Sf9 cells were infected with AcVGspy virus at an moi of 1 for 30 hours at 28 °C for labelling. Then, cells were washed twice with Grace's Insect Medium. QD605-SpyCatcher in Grace's medium containing 10% FBS was filtered through a 0.45 μm filter before incubated with cells to prevent potential bacterial contamination. Cells were observed for QD binding or fixed with 4% paraformaldehyde. For virus labelling, at 3 hours post incubation, cells were washed carefully to remove unbound QDs and cultured with

fresh medium. The supernatant was harvest at 18 hours post QD incubation and concentrated as described before.

Fluorescence imaging and analysis: Sf9 cells seeded in a glass-bottom cell Imaging dish (170 µm, 35*10 mm) for fluorescence imaging using the UltraView VOX confocal system (PerkinElmer) with an inverted microscope (Nikon). For imaging QD labelling of GP64spy on cell membrane, infected cells were either fixed with 4% paraformaldehyde or subjected to confocal microscopy imaging directly after QD605-SpyCatcher labelling. For living cell imaging, Sf9 cells were infected at a high moi (>100) at 4 °C for 15 minutes. Next, 1 ml of Grace's medium containing 10% FBS prewarmed at 28 °C was added to the dish just before living cell imaging. During imaging, cells were kept in a heated chamber at 27 °C. The infection process was recorded with the Volocity software (PerkinElmer). For line profile and co-localization analysis, image-pro-plus software was used.

1. F. Zhang, Z. Zheng, S. L. Liu, W. Lu, Z. Zhang, C. Zhang, P. Zhou, Y. Zhang, G. Long, Z. He, D. W. Pang, Q. Hu and H. Wang, *Biomaterials*, 2013, **34**, 7506-7518.
2. B. Zakeri, J. O. Fierer, E. Celik, E. C. Chittock, U. Schwarz-Linek, V. T. Moy and M. Howarth, *Proceedings of the National Academy of Sciences of the United States of America*, 2012, **109**, E690-697.

2. Additional figures

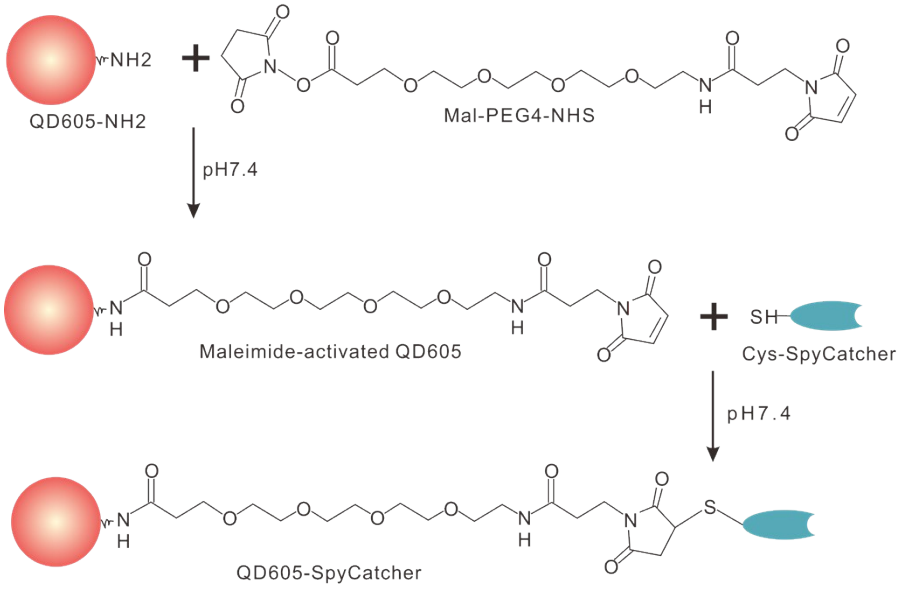

Figure S1. Surface modification of QD605-NH2 with Cys-SpyCatcher protein. QD605-NH2 was first reacted with Mal-PEG4-NHS bifunctional linker to generate maleimide-activated QD605 and then conjugated with Cys-SpyCatcher proteins through maleimide-thiol reaction to produce QD605-SpyCatcher.

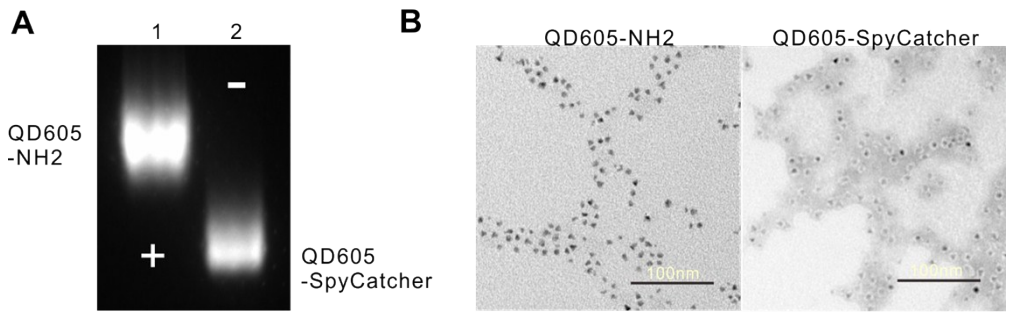

Figure S2. Characterization of QD605-SpyCatcher. **A:** Agarose gel electrophoresis of QD605-NH2 (lane 1) and QD605-SpyCatcher (lane 2). **B:** TEM images of QD605-NH2 and QD605-SpyCatcher. Scale bar: 100 nm.

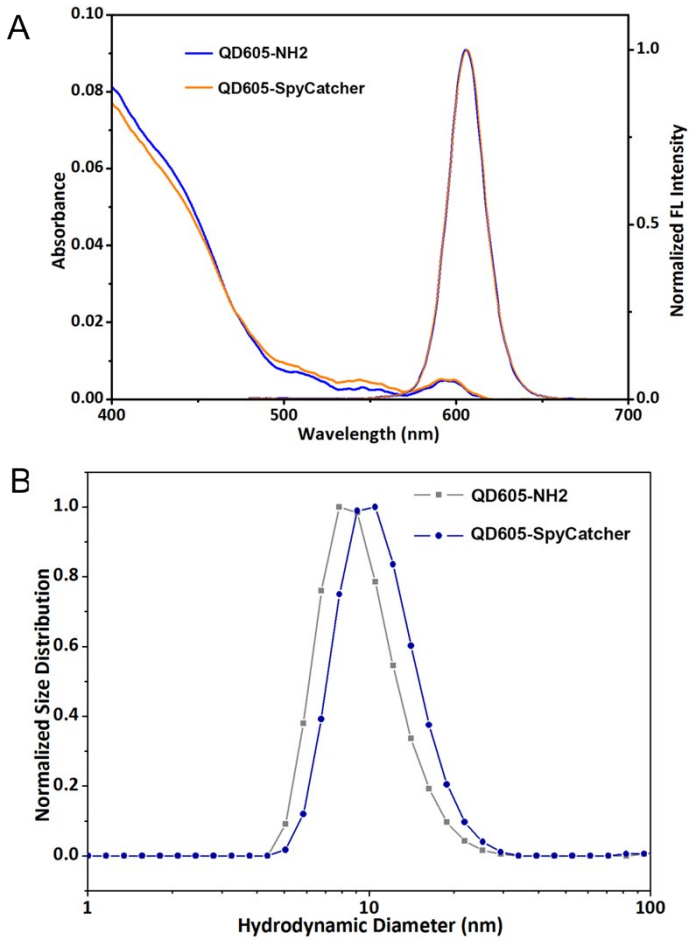

Figure S3. Spectra and hydrodynamic diameter analysis of QD605-NH2 and QD605-SpyCatcher. A: Normalized UV and PL spectra of QD605-NH2 (blue) and QD605-SpyCatcher (red). B: Hydrodynamic diameter of QD605-NH2 (grey) and QD605-SpyCatcher (blue).

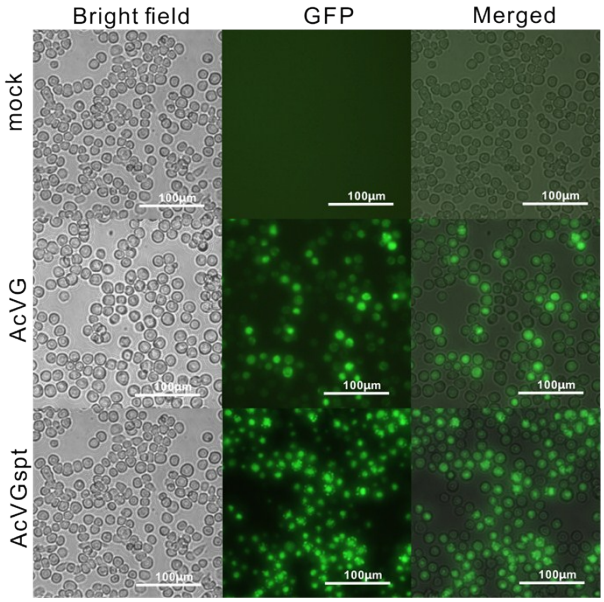

Figure S4. Cells infected with recombinant baculovirus AcVGspy and AcVG. GFP expression and CPE could be observed in AcVGspy and AcVG infected Sf9 cells. Images were taken by using a microscope (OLYMPUS). Scale bar: 100 μ m.

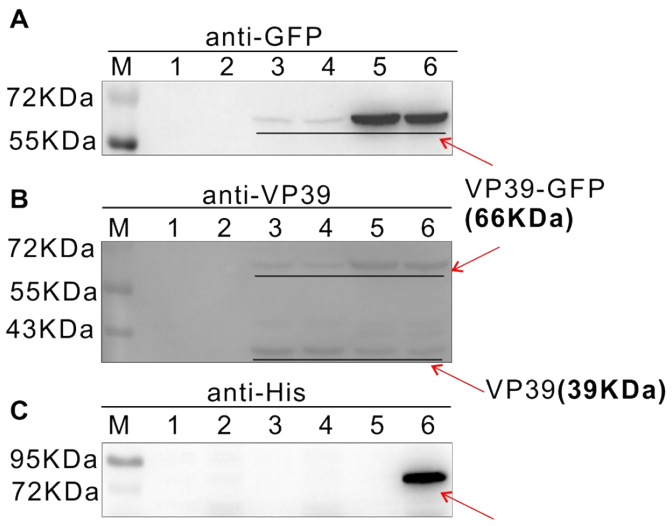

Figure S5. Detection of GP64spy through formation of GP64spy-SpyCatcher complex. Sf9 cell lysate, AcVG infected cell lysate and AcVGspy infected cell lysate were directly boiled (channel **1,3 and 5**) or incubated with His-SpyCatcher proteins (channel **2,4 and 6**) before subjected to Western blotting to detect GFP-VP39 fusion proteins **(A and B)** or conjugated SpyCatcher-GP64 protein complex (**C and D**). **M:** marker.