Electronic Supplementary Material (ESI)

Internalization of pseudorabies virus via macropinocytosis analyzed by quantum dot-based single-virus tracking

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Supporting Figures

S. 1 Internalization of pseudorabies virus (PrV) associates with actin-rich protrusions

The diameter of vesicles that containing enveloped PrV was calculated by Nano Measurer 1.2 software and analyzed by origin software. As shown in Fig. S1, the diameter of all vesicles is above 0.2 μm and most of them is about 0.5 μm, which is accordant with macropinosomes, indicating that enveloped PrV was internalized into HeLa cells in macropinosomes.

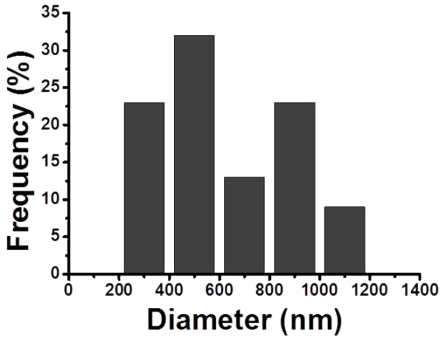

Fig. S1 The statistics of diameter of vesicles containing PrV (n = 20).

HeLa cells were transfected with enhanced green fluorescence protein (EGFP)-lifeact-peptide plasmid to label actin with EGFP. Then the actin of HeLa cells was immunofluorescence labeled with Cy3. Fig. S2A showed that EGFP labeled actins colocalized well with Cy3 immunofluorescence labeled actins, suggesting that actins could be labeled efficiently by transfecting plasmid.

Actin of HeLa cells was labeled by transfecting EGFP-lifeact-peptide plasmid. Then the cells were incubated with QDs-PrV. Confocal images (Fig. S2B) showed that after QDs-PrV incubating with HeLa cells for 10 min at 4 °C, most QDs-PrV attached to the actin-rich protrusions on the surface of plasma membranes, indicating that PrV interacted with actin-rich protrusions.

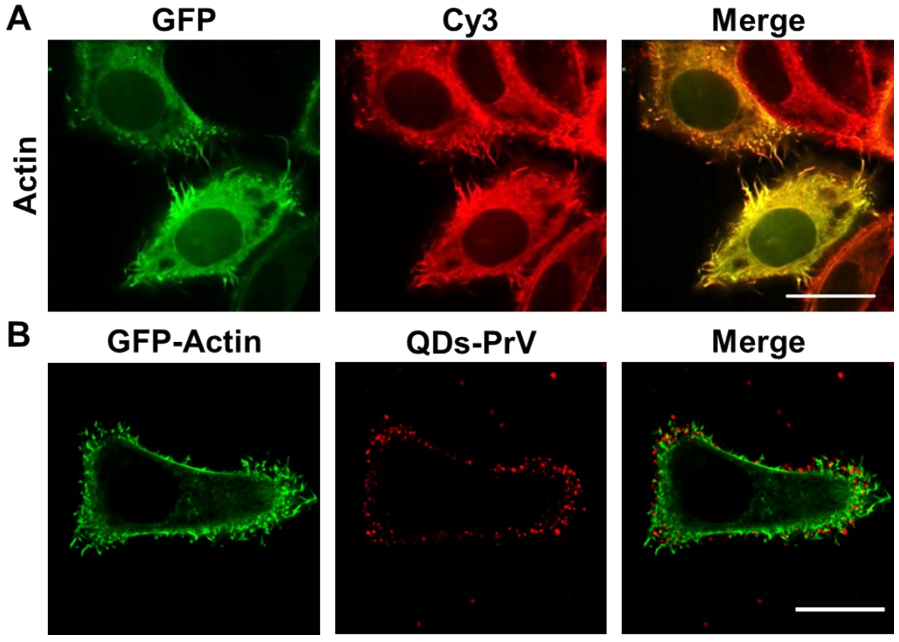

Fig. S2 (A) Fluorescent images of enhanced green fluorescence protein (EGFP) labeled actins (green), Cy3 immuno-labeled actins (red) and their merge (yellow). (B) Fluorescent images of EGFP labeled actins (green), QDs-PrV (red) and their merge (yellow). Scale bar: 20 μm.

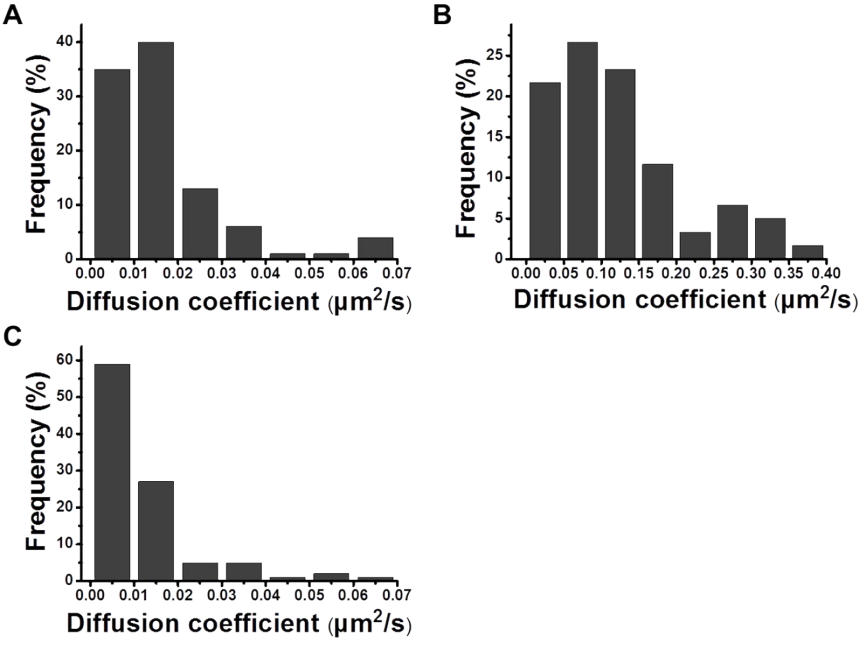

Fig. S3 Statistical analysis of the diffusion coefficient of QDs-PrV attaching at actin-rich protrusions (A, n = 100), moving along the actin-rich protrusions (B, n = 60), and moving at the near plasma membrane region of a living HeLa cell (C, n = 100).

HeLa cells were treated with cytochalasin D (cyto D), which is a cell-permeable and potent inhibitor of actin polymerization, and then infected with QDs-PrV to determine the dependence of PrV internalization on actin. As shown in Fig. S4, only few of QDs-PrV signals (red) were observed in the cytoplasm of HeLa cells treated with cyto D while tens of QDs-PrV signals could be found in the cytoplasm of untreated HeLa cells. The statistics analysis suggested that the number of QDs-PrV in cyto D treated HeLa cells decreased significantly (n=100, P<0.01), indicating that the internalization of PrV was dependent on actins polymerization.

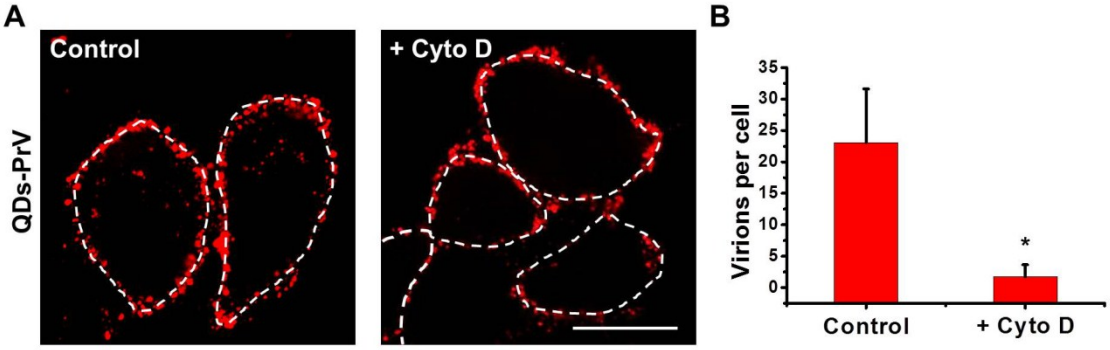

Fig. S4 (A) Confocal images of QDs-PrV infecting cyto D treated HeLa cells. Scale bar: 20 μm. (B) Statistics of the internalized QDs-PrV in cyto D treated/untreated HeLa cells (n = 100, error bars represent S.D.). Asterisks indicate significant difference from controls (P < 0.01, t test).

S. 2 PrV is internalized into HeLa cells in macropinosomes

Fluorescein isothiocyanate–dextran (average mole weight 70000, FITC-DEX70), a fluid phase marker, is demonstrated to be internalized by macropinocytosis.[1] As shown in Fig. S5, the average fluorescence intensity of FITC-DEX70 in a HeLa cell increased as the hours post-infection (h. p. i.) of PrV increased, suggesting the infection of PrV induced a

significant increase of fluid uptake.

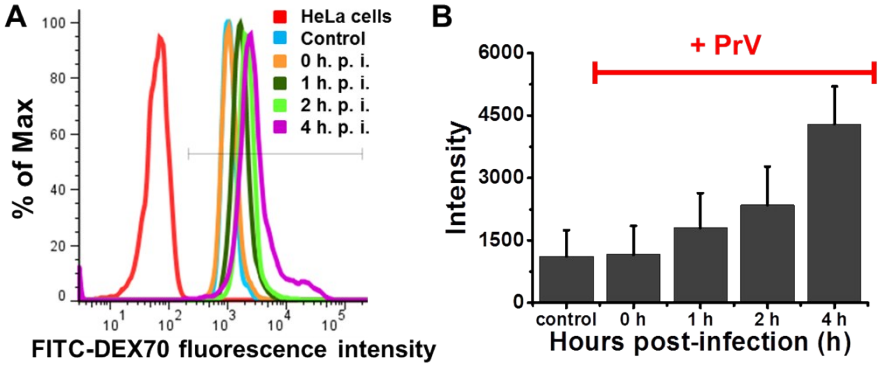

Fig. S5 HeLa cells were infected with PrV for different hours post-infection (h.p.i.) and then incubated with FITC-DEX70 for 1 h. (A) Percentage and (B) the mean fluorescence intensity of FITC-DEX70-positive cells were analyzed by flow cytometry (n = 10000, error bars represent S.D.). HeLa cells were infected with purified PrV (MOI of 1) for different h.p.i. and then incubated with 2 mg/mL FITC-DEX70 at 37 °C for 1 h. After being extensively washed with 1×PBS for three times, the prepared samples were detected by flow cytometer (FACSAria III BD).

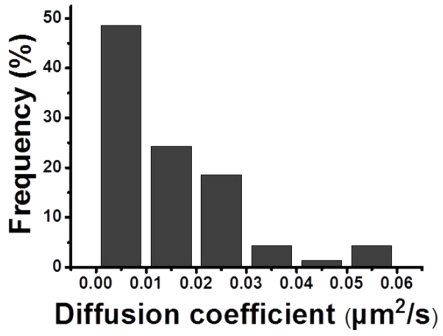

Fig. S6 Statistical analysis of the diffusion coefficient of QDs-PrV being internalized into HeLa cells together with FITC-DEX70 (n = 70).

S. 3 Typical regulators involved in macropinocytosis play important roles in the internalization of PrV

Wortmannin and LY294002, inhibitors of PI3K; rottlerin, inhibitor of PKC; IPA-3,

inhibitor of Pak1; and 5-(N, N-Dimethyl) amiloride hydrochloride (EIPA), inhibitor of NHE were used in corresponding assays, respectively. MTT assay showed that the viability of HeLa cells incubated with inhibitors at working concentrations for 3 h was similar to that of HeLa cell cultured in DMEM (Fig. S7), demonstrating that working concentrations of inhibitors have no significant influence on the viability of HeLa cells during experiments.

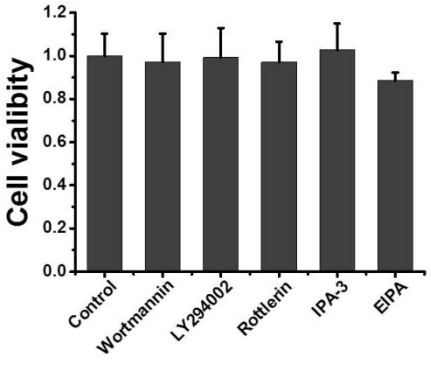

Fig. S7 MTT assay of HeLa cells cultured at working concentration of macropinocytosis inhibitors for 3 hours (n = 9).

Fig. S8A shows that the uptake of FITC-DEX70 in HeLa cells treated with corresponding inhibitors decreases obviously. Moreover, confocal images (Fig. S8B) shows that few signals of FITC-DEX70 can be observed in the cytoplasm of HeLa cells treated with inhibitors while obvious FITC-DEX70 signals can be seen in control. Combining the results, all these inhibitors inhibited the uptake of FITC-DEX70, suggesting these inhibitors inhibited macropinocytosis effectively since FITC-DEX70 is demonstrated to be internalized by macropinocytosis.

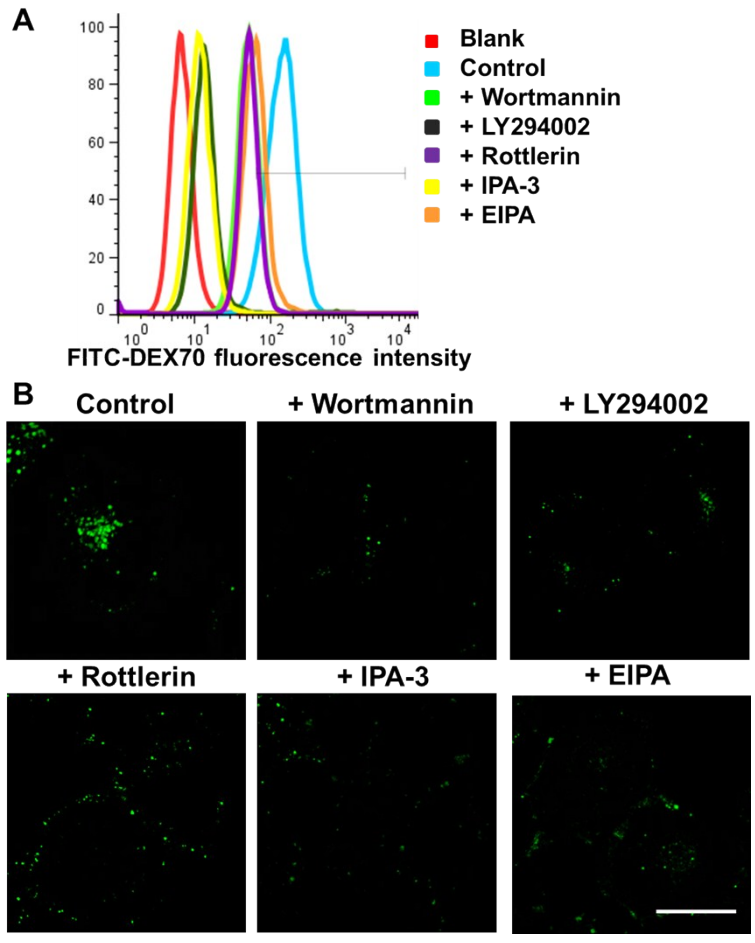

Fig. S8 Internalization of FITC-DEX70 into HeLa cells treated with corresponding inhibitors. Flow cytometry of HeLa cells treated with different corresponding inhibitors (A) incubated with FITC-DEX70 (n = 10000). (B) Confocal images of corresponding inhibitors treated HeLa cells incubated with FITC-DEX70. Scale bar: 20 μm. HeLa cells were pretreated with corresponding inhibitors at 37 °C, 5% CO_2 for 30 min or 1 h and then incubated with 2 mg/mL FITC-DEX70 at 37 °C, 5% CO_2 for 1 h, respectively. The prepared samples were detected by confocal microscope or flow cytometer. The results were analyzed by Imaging-Pro-Plus (IPP) software and Flowjo software.

In order to demonstrate the relationship between internalization of PrV and typical regulators involved in macropinocytosis. HeLa cells treated with corresponding inhibitors were infected with PrV encoding a GFP reporter gene. As shown in Fig. S9A, the

percentage of GFP-positive cells treated with corresponding inhibitors reduced. Moreover, the average fluorescence intensity of GFP in each cell decreased obviously (Fig. S9B), indicating that the number of virions that were internalized into infected cells decreased significantly.

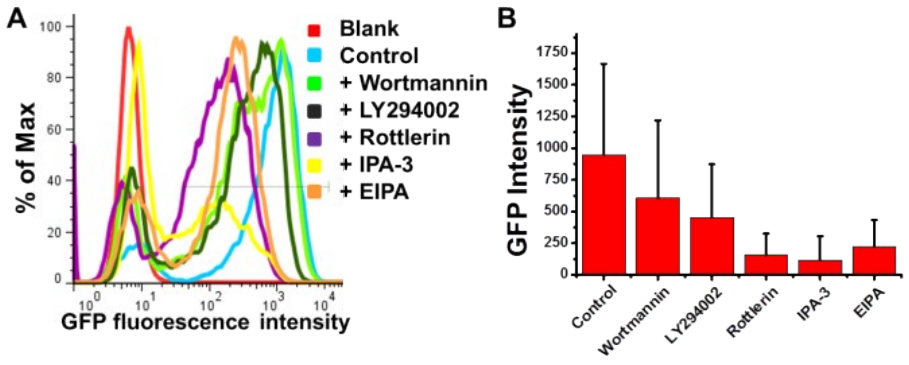

Fig. S9 Percentage (A) and the mean fluorescence intensity (B) of GFP-positive cells were analyzed by flow cytometry (n = 10000, error bars represent S.D.). HeLa cells seeded in 12-well plates in advance were pretreated with inhibitors at 37 °C, 5% CO_2 for 30 min or 1 h, respectively. Then, the cells were attached by PrV at MOI of 1 on ice for 1 h. Then, the supernatants were replaced by 1 mL DMEM containing inhibitors, respectively. The infected cells were incubated at 37 °C, 5% CO_2 for 1 h to make virus enter cells and then treated with 1 mL of sodium citrate buffer (40 mM sodium citrate, 10 mM KCl and 135 mM NaCl, pH 3.0) for 1 min at room temperature for inactivation of virus. At last, cells were cultured in DMEM supplemented with 2% (v/v) FBS at 37 °C, 5% CO_2 for 12 h, respectively. The expression of reporter gene GFP was detected by flow cytometer. The results were analyzed by Flowjo software.

To confirm the roles of these Rho GTPases in macropinocytosis, Rhodamine B-DEX70 (RhoB-DEX70) was used as control. Compared with the signals of RhoB-DEX70 in HeLa cells expressing Rac1 WT and Cdc42 WT, the signals of RhoB-DEX70 decreased significantly in HeLa cells expressing dominant negative form of Rac1 and Cdc42 (Rac1

DN and Cdc42 DN), suggesting the expression of Rac1 DN and Cdc42 DN could disturb macropinocytosis process (Fig. S10A and B). Moreover, the internalization of RhoB-DEX70 in HeLa cells expressing RhoA WT and RhoA DN were similar, indicating the expression of RhoA was irrelevant to macropinocytosis process (Fig. S10C).

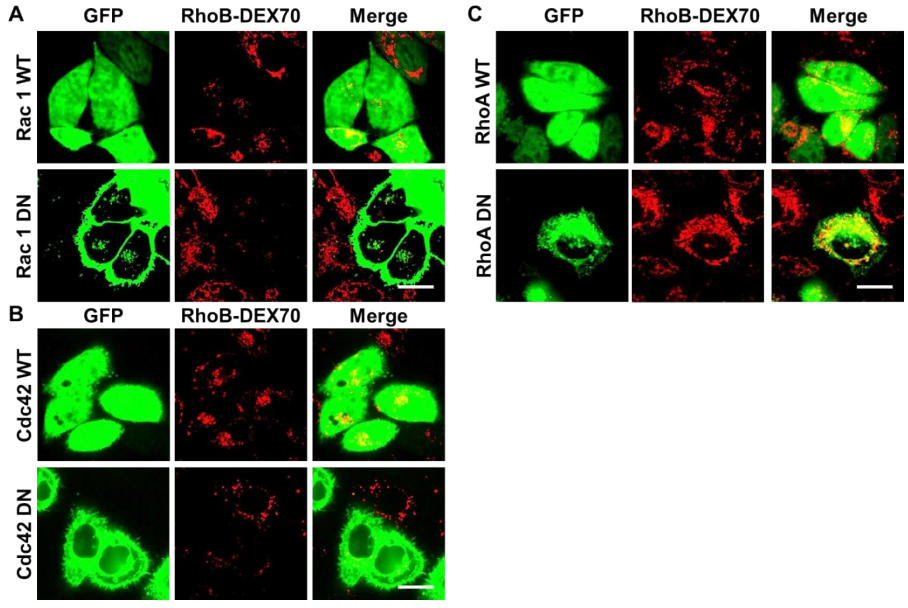

Fig. S10 Confocal images of HeLa cells transfected with a wild type (top) or dominant-negative (bottom) form of Rac1 (A), Cdc42 (B) or RhoA (C) plasmid and then incubated with 2 mg/mLRhoB-DEX70. Scale bar: 20 μm. HeLa cells were transfected with the plasmids encoding wild type or dominant negative form of Rac1, Cdc42 or RhoA, respectively. The cells were incubated with 2 mg/mL RhoB-DEX70 at 37 °C, 5% CO_2 for 1 h. After the redundant RhoB-DEX70 was washed out, the prepared samples were observed by microscope and the results were analyzed by IPP software.

S. 4 Internalization of PrV is irrelevant to dynamin, clathrin or caveolin

To verify the function of corresponding inhibitors, the internalization of tetramethylrhodamine conjugated transferrin (TMR-transferrin, clathrin-mediated endocytosis marker), Alexa-Fluor 561 labeled cholera toxin subunit B (Alexa-Fluor 561-CtxB, caveolin-mediated endocytosis marker) and FITC-DEX70 into inhibitors treated

HeLa cells were tested, respectively. MTT assay (Fig. S11A) showed that the viability of HeLa cells incubated with inhibitors at working concentration for 3 h was similar to that of HeLa cell cultured in DMEM, indicating that the treatment of HeLa cells with inhibitors at working concentration had no effect on the viability of HeLa cells. As shown in Fig. S11B, the internalization of TMR-transferrin was inhibited effectively by dynasore, OcTMAB, or chlorpromazine. Meanwhile, the internalization of Alexa-Fluor 561-CtxB was inhibited effectively by dynasore, OcTMAB or genistein (Fig. S11C). Moreover, according to the results of flow cytometry and confocal images, none of these inhibitors influence the internalization of FITC-DEX70 (Fig. S10). The results imply that the inhibitors block dynamin-, clathrin- and caveolin-mediated endocytosis, respectively. However, flow cytometry and confocal images (Fig. S12) show that none of these inhibitors influence the internalization of FITC-DEX70, indicating the inhibitors are irrelevant to macropinocytosis process.

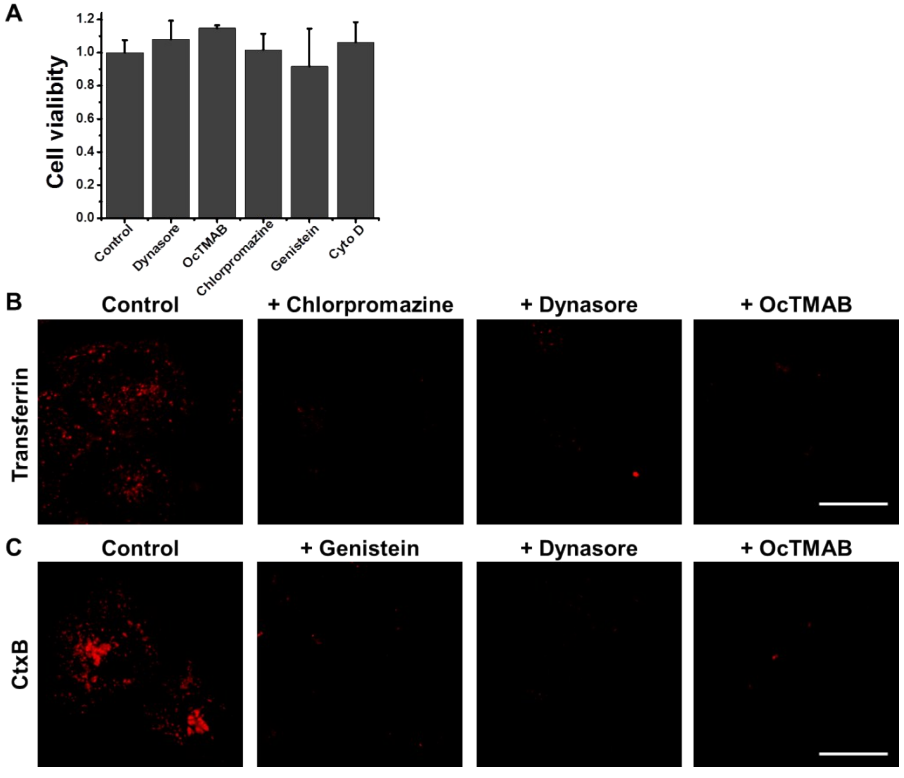

Fig. S11 (A) MTT assay of HeLa cells cultured in DMEM containing working

concentration of dynamin, clathrin, caveolin inhibitors and cyto D for 3 hours (n = 9). HeLa cells were cultured in 96-well plates at 37 °C, 5% CO_2 in advance. Then, cells were incubated in DMEM containing corresponding inhibitors at 37 °C, 5% CO_2 for 3 h. Subsequently, media were replaced by 200 μL of 0.5 mg/mL 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT). After the cells were incubated at 37 °C, 5% CO_2 for 4 h, the liquids were removed and 200 μL of dimethyl sulfoxide was added. The UV absorbance at 490 nm was measured. (B-C) Confocal images of dynamin, clathrin or caveolin inhibitors treated HeLa cells incubated with TMR-transferrin or Alexa-Fluor 561-CtxB. HeLa cells were pretreated with inhibitors at 37 °C, 5% CO_2 for 30 min or 1 h and then treated with 25 μg/mL TMR-transferrin or Alexa-Fluor 561-CtxB at 4 °C for 10 min, shifted to 37 °C for 20 min for internalization. After the unbound TMR-transferrin or Alexa-Fluor 561-CtxB were washed out, the samples were observed by confocal microscope. The results were analyzed by IPP software. Scale bar: 20 μm.

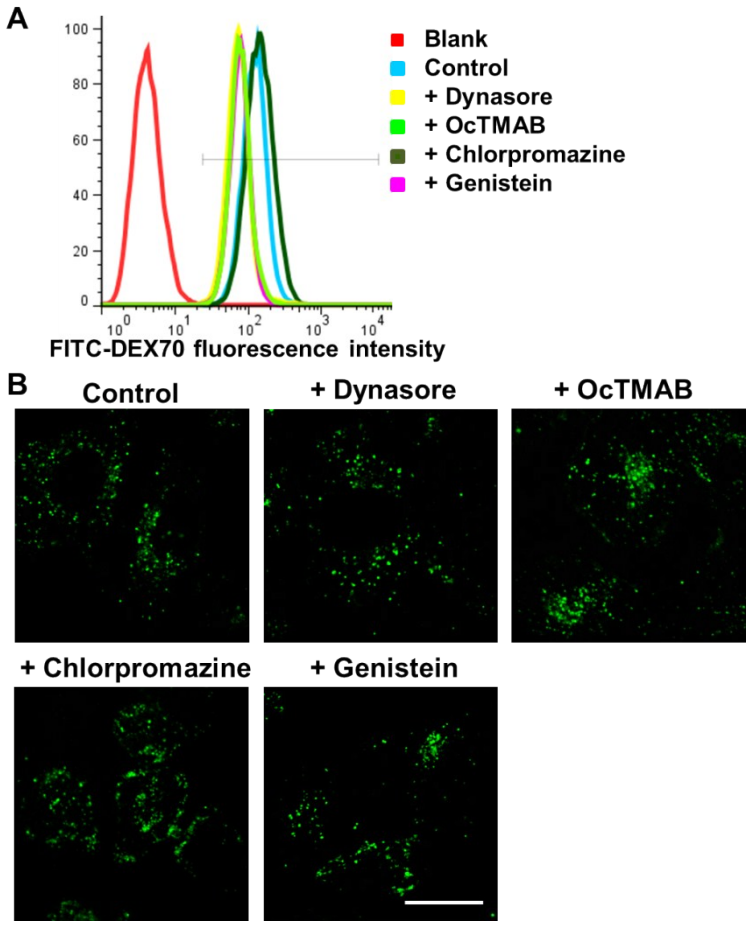

Fig. S12 (A) Flow cytometry of dynamin, clathrin or caveolin inhibitors treated HeLa cellsincubated with FITC-DEX70 (n = 10000). (B) Confocal images of dynamin, clathrin or caveolin inhibitors treated HeLa cells incubated with 2 mg/mL FITC-DEX70. HeLa cells were pretreated with inhibitors at 37 °C, 5% CO_2 for 30 min or 1 h and then incubated with 2 mg/mL FITC-DEX70 at 37 °C, 5% CO_2 for 1 h, respectively. The prepared samples were detected by confocal microscope and flow cytometer. The results were analyzed by IPP software and Flowjo software. Scale bar: 20 μm.

The expression of reporter gene GFP in inhibitors treated cells exhibited no obvious decrease (Fig. S13), indicating that inhibitors of dynamin, clathrin or caveolin did not influence the internalization of PrV.

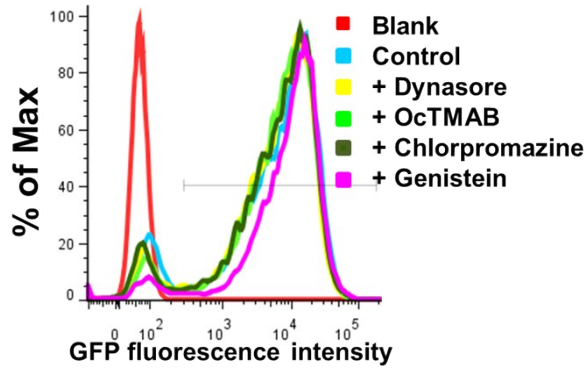

Fig. S13 Flow cytometry of PrV encoding a GFP reporter gene infected HeLa cells treated with dynamin, clathrin or caveolin inhibitors (n = 10000). HeLa cells seeded in 12-well plates in advance were pretreated with inhibitors at 37 °C, 5% CO_2 for 30 min or 1 h, respectively. The cells were attached by PrV at MOI of 1 on ice for 1 h and then the supernatants were replaced by 1 mL DMEM containing corresponding inhibitors, respectively. The infected cells were incubated at 37 °C, 5% CO_2 for 1 h to make virus enter cells and then treated with 1 mL of sodium citrate buffer for 1 min at room temperature for inactivation of virus. At last, cells were cultured in DMEM supplemented with 2% (v/v) FBS at 37 °C, 5% CO_2 for 12 h, respectively. The expression of reporter gene GFP was detected by flow cytometer. The results were analyzed by Flowjo software.

Experimental Section

Reagents and antibodies. Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from GIBCO. Biotin functionalized phospholipid 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotinyl (polyethylene glycol) 2000] (DSPE-PEG2000-biotin) was purchased from Avanti Polar Lipids. Streptavidin-conjugated quantum dots (SA-QDs) were purchased from Wuhan Jiayuan Quantum Dots Co. Ltd. Primary antibody against SNX5, dynasore and OcTMAB were purchased from Abcam. Dylight 488 labeled secondary antibody was purchased from EarthOx. Wortmannin, TMR-transferrin and Alexa-Fluor 561-CtxB were purchased from Life Technologies. FITC-DEX70, RhoB-DEX70, LY294002, IPA-3, rottlerin, cyto D, EIPA, and chlorpromazine were purchased from Sigma. Genistein was obtained from TCI. Plasmids, pcDNA3-EGFP-Rac1-T17N, pcDNA3-EGFP-Cdc42-T17N, pcDNA3-EGFP-RhoA-T19N, were gifts from Gary Bokoch (Addgene plasmid #12982, #12976 and #12967).[2] Plasmid EGFP-lifeact-peptide was kindly provided by Prof. Zongqiang Cui in Wuhan Institute of Virology, Chinese Academy of Sciences.

Cell culture and transfection. PK15 and HeLa cells were cultured in DMEM supplemented with 10% (v/v) FBS at 37 °C, 5% CO_2. Biotinylated PK15 cells (B-PK15) were produced by culturing PK15 cells in DMEM containing 0.025 mg/mL DSPE-PEG2000-biotin and 10% (v/v) FBS at 37 °C, 5% CO_2. Plasmids encoding EGFP-lifeact-peptide, mEGFP-Rac1, mEGFP-Cdc42, mEGFP-RhoA, pcDNA3-EGFP-Rac1-T17N, pcDNA3-EGFP-Cdc42-T17N, pcDNA3-EGFP-RhoA-T19N, AcGFP1-dynamin 2, AcGFP1-clathrin or AcGFP1-caveolin were used to label actin, wild type Rac1 (Rac1 WT), wild type Cdc42 (Cdc42 WT), wild type RhoA (RhoA WT), dominant negative Rac1 (Rac1 DN), dominant negative Cdc42 (Cdc42 DN), dominant negative RhoA (RhoA DN), dynamin, clathrin or caveolin, respectively. HeLa cells were planted in 24-well plates at 37 °C, 5% CO_2 in advance and then transfected with corresponding plasmid by using Lipofectamine LTX and PLUS reagents (Invitrogen) according to the manufacturer's

instructions. After 24 h, HeLa cells expressing GFP labeled actin, MTs, Rac1 WT, Cdc42 WT, RhoA WT, Rac1 DN, Cdc42 DN, RhoA DN, dynamin, clathrin or caveolin were planted into 35 mm glass based dish for confocal microscope observation.

MTT assay. HeLa cells were planted in 96-well plates at 37 °C, 5% CO_2 in advance. Then, cells were incubated with working concentration of corresponding inhibitors at 37 °C, 5% CO_2 for 3 h. Afterwards, medium was replaced by 200 μL of 0.5 mg/mL 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT). After incubating at 37 °C, 5% CO_2 for 4 h, the liquid was removed and 200 μL of dimethyl sulfoxide (DMSO) was added. The UV absorbance at 490 nm was measured. All samples were performed in nonuplicate.

Replication, purification and QDs-labeling of PrV. Wild type PrV was kindly provided by Prof. Hanzhong Wang in Wuhan Institute of Virology, Chinese Academy of Sciences. Biotinylated PrV was propagated in B-PK15 cells in DMEM supplemented with 2% (v/v) FBS at 37 °C, 5% CO_2 for 48 h. After two freeze-thaw cycles, the infected B-PK15 cells were centrifuged at 5000 rpm for 30 min to remove cell debris. Then, the supernatant was concentrated by ultracentrifugation at 30000 rpm for 2.5 h in a Type 45 rotor (Beckman). The precipitation was resuspended in 1×PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) and dispersed extensively by vortex for 10 min. Afterwards, virus was incubated with 20 nM SA-QDs for 1 h on ice, and then purified by 30%-55% sucrose density gradient centrifugation at 26000 rpm for 2.5 h in an SW41 rotor (Beckman). The QDs-PrV was collected under UV excitation, resuspended in 1×PBS and centrifuged at 25000 rpm for 3 h to remove sucrose. QDs-PrV was resuspended in 1×PBS and then filtered through a 0.22 μm film (Millipore) for further use.

Transmission electron microscopy (TEM). Purified PrV (MOI of 50) was added to HeLa cells and incubated on ice for 1 h for attachment. Cells were washed three times with 1×PBS to remove redundant virus and then fixed with the fixed buffer (0.1 M sodium cacodylate buffer containing 2% (v/v) glutaraldehyde) on ice for 15 min. After being washed with the fixed buffer thrice, the infected cells were scraped and suspended in the

fixed buffer. Ultra-thin section samples were prepared by Wuhan Institute of Virology and observed with cryoelectron microscope (Tecnai G20 TWIN, FEI).

Fluorescence colocalization. HeLa cells planted in a 35 mm glass based dish in advance were incubated with QDs-PrV and 2 mg/mL FITC-DEX70 at 37 °C, 5% CO_2 for 1 h. Subsequently, the cells were fixed with 4% (w/v) paraformaldehyde for 20 min at room temperature and then observed by confocal microscope (Andor Revolution XD).

SNX5, localized in newly formed macropinosomes, may act to distort the plasma membrane and thereby generate a macropinocytic pit, which then buds off from plasma membrane to form a macropinosome.[3] Cells attached by QDs-PrV were incubated at 37 °C, 5% CO_2 for 1 h and fixed with 4% (w/v) paraformaldehyde for 20 min at room temperature. Then the cells were blocked with 1×PBS containing 5% (w/v) BSA for 30 min at 37 °C. After being excessively washed with 1×PBS, the cells were incubated with the primary antibody against SNX5 at 37 °C for 1 h. To remove redundant primary antibody, the cells were shaken in 1×PBS for 5 min at room temperature thrice. Then the cells were incubated with Dylight 488 conjugated secondary antibody at 37 °C for 40 min and washed thrice with 1×PBS. The prepared samples were observed by confocal microscope.

Inhibitors treatment assay. Cyto D, wortmannin, LY294002, rottlerin, IPA-3, EIPA, dynasore, OcTMAB, chlorpromazine or genistein were dissolved in DMSO and then diluted with 1×PBS to prepare working solutions with final concentrations of 20 nM, 300 nM, 50 μM, 50 μM, 25 μM, 50 μM, 80 μM, 30 μM, 30 μM or 200 μM, respectively. HeLa cells seeded in 35 mm glass based dish in advance were pretreated with corresponding inhibitors at the working concentration for 30 min, 1 h, 30 min, 30 min, 30 min, 30 min, 30 min, 30 min, 1 h or 1 h prior to the attachment of QDs-PrV at 4 °C for 10 min. Subsequently, the cells were incubated at 37 °C for 1 h with the presence of corresponding inhibitors. The prepared samples were observed by confocal microscope.

Fluorescence imaging. HeLa cells expressing EGFP labeled actin, EGFP labeled Rac1 WT, EGFP labeled Cdc42 WT, EGFP labeled RhoA WT, EGFP labeled Rac1 DN, EGFP labeled

Cdc42 DN, EGFP labeled RhoA DN, GFP labeled dynamin, GFP labeled clathrin or GFP labeled caveolin were seeded in 35 mm glass based dishes in advance and then incubated QDs-PrV at 4 °C for 10 min. After washing redundant QDs-PrV, the infected cells were incubated at 37 °C for 1 h, except for the cells expressing EGFP labeled actin. The prepared samples were observed by a spinning-disk confocal microscope. Dylight 488/GFP/FITC, TMR/Alexa Fluor 561/Cy3/RhoB and 705 nm SA-QDs were excited by 488 nm, 561 nm and 488 nm laser (DPSS, USA), respectively. The emission signals were separated by 525/50 nm, 617/73 nm and 685/40 nm filters (Chroma) and recorded by an EMCCD (Andor iXon DU897U single photon detector).

Statistical analyses. The edge of cells were identified by bright field image and then the number of internalized virions were counted by Imaging-Pro-Plus (IPP) software (Media Cybernetics). The colocalization efficiency of fluorescent signals from two channels was quantified by intensity correlation analysis performed by Image J. The value of tMr suggested the percentage of red signals colocalized with green signals in the image. The ICQ value indicated the correlation intensity of two channels and ranged from -0.5 to 0.5 (+0.1 to +0.5 implies a strong covariance). All trajectories of QDs-PrV were tracked and performed with IPP software. MSD was calculated by the user-written program with Matlab.[4] The linear is the fit to MSD = $4D\tau$. The upward curve is the fit to MSD = $4D\tau + (V\tau)^2$. The downward curve is the fit to MSD = $4D\tau^{\alpha}$ (D is the diffusion coefficient, V is the mean velocity of the particle and α is a constant of less than 1).

Movie legends

Movie S1. QDs-PrV (red) attached at actin-rich protrusions (green) of a living HeLa cell. HeLa cells expressing EGFP-actin were seeded in a 35 mm glass based dish and QDs-PrV was added into the dish directly. The images were recorded every 1 s by EMCCD.

Movie S2. QDs-PrV (red) was moving along the actin-rich protrusions (green) of a living HeLa cell. HeLa cells expressing EGFP-actin were seeded in a 35 mm glass based dish and QDs-PrV was added into the dish directly. The images were recorded every 2 s by EMCCD.

Movie S3. QDs-PrV (red) was moving at the near plasma membrane region (green) of a living HeLa cell. HeLa cells expressing EGFP-actin were seeded in a 35 mm glass based dish and QDs-PrV was added into the dish directly. The images were recorded every 2 s by EMCCD.

Movie S4. QDs-PrV (red) was internalized into a cell together with FITC-DEX70 (green). HeLa cells were incubated with QDs-PrV at 4 °C for 10 min. After washing the redundant viruses, the infected cells were incubated at 37 °C for 30 min. The images were recorded every 2 s by EMCCD.

References

1. L. Li, T. Wan, M. Wan, B. Liu, R. Cheng, R. Zhang, *Cell Biol. Int.* 2015, **39**, 531-539.
2. M. C. Subauste, M. Von Herrath, V. Benard, C. E. Chamberlain, T. H. Chuang, K. Chu, G. M. Bokoch, K. M. Hahn, *J. Biol. Chem.* 2000, **275**, 9725-9733.
3. J. P. Lim, J. T. Wang, M. C. Kerr, R. D. Teasdale, P. A. Gleeson, *BMC Cell Biol.* 2008, **9**, 58.
4. M. J. Saxton, *Biophys. J.* 1997, **72**, 1744-1753.