Supporting information

His-tag based *in situ* labelling of progeny virus for real-time single virus tracking in living cells

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1. Experimental Details

Preparation of QD-NTA-Ni^{2+}. The QDs-NTA-Ni^{2+} were prepared according to reported method with some modifications.[1] Briefly, carbonyl-modified CdSe/ZnS QDs (QD-COOH, Wuhan Jiayuan Co., China) were activated by new prepared N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) in pH 7.4 phosphate buffer (PB) solution for 30 min at room temperature. Then, 4 mM *Nα*,*Nα*-bis(carboxymethyl)-L-lysine hydrate (NTA-lysine, Sigma) solution was incubated with the activated QD-COOH for 4 h at room temperature with the presence of Zonyl® FSN fluorosurfactant (FSN, Sigma) to prevent QDs aggregation. Then, 10 mM $NiCl_2$ solution were mixed with QD-NTA for another 2 h for complexing Ni^{2+}. After ultrafiltration to remove the redundant reagents, the QD-NTA-Ni^{2+} were suspended in pH 7.4 phosphate buffer saline for further use.

Cell culture and virus propagation. HEp-2 cells were maintained in a 5% CO_2 environment with RPMI 1640 medium (Hyclone) supplemented with 10% fetal boving serum (FBS, Gibco), 100 U/mL penicillin G and 100 ug/mL streptomycin sulfate. Human Respiratory Syncytial Virus strain Long (Guangzhou Biotest bioengineering Co., LTD, China) was propagated in monolayer HEp-2 cells in RPMI 1640 medium with 2% FBS for 2-3 days. Then, the viruses were harvested after repeat freeze-thaw cycles of cells and centrifugation.

Preparation of QD-labelled RSV. For direct labelling, the carboxyl group of the polypeptide (HHHHHHTSTSGG, His6) (Beijing Zhongke Yaguang biological technology Co., LTD, China) was firstly activated by the new prepared EDC/NHS for 15 min in pH 6.0 2-(N-morpholino)ethanesulfonic acid (MES) buffer solution at room temperature, then incubated with viruses for 2 h at 4 ^{o}C. The redundant His6 peptide was removed by ultrafiltration. For *in situ* progeny virus labelling, since the viruses were labelled during propagation, the HEp-2 cells were firstly inoculated with RSV. After 48 h post-infection, cells were incubated with 1 mg/mL activated His6 peptide for 30 min. Then, the cells were subjected to 2 rounds of freeze-thaw cycles to release progeny viruses labelled with His tag. The cell debris was removed by centrifugation at 3000 rpm at 4 ^{o}C for 10 min. The labelled viruses were purified by ultracentrifugation (Beckman 70Ti) at 78000×g for 1 h, over a sucrose cushion (30% sucrose in 0.1 M sodium chloride, 0.01 M Tris-HCl, 0.001 M EDTA, 1 M urea, pH = 7.5).

Virus titer quantification. The infection titers of normal RSV and labelled RSV were determined by 50% tissue culture infective dose (TCID50). Initially, HEp-2 cells were cultured in 96-well plates with RPMI 1640 medium supplemented with 2% FBS for 24 h. Then, virus samples were serially diluted with medium with dilution factor 10 and introduced to the cell monolayer. After inoculation for 2 h at 37 ^{o}C, inoculum was removed, and the infected cells were cultured with RPMI 1640 medium supplemented with 2% FBS for 6-8 days. Cell monolayers were rinsed with PBS, fixed with 2.5% glutaraldehyde and stained by 0.5% neutral red. Dead cells and infected cells with evident cytopathic effect (CPE) were easily washed off and couldn't be stained by neutral red. The number of positive well of ever dilution was counted, and the TCID50 was calculated by Formula of Reed-Muench: logTCID50 = log(dilution above 50% CPE) − [(% next above 50%) − 50%]/[(% next above 50%) − (% next below 50%)] × log(dilution factor). TCID50/mL is then calculated as 1/(TCID50 × volume/mL per well).

Immunofluorescence staining and analysis. HEp-2 cells were cultured in 35 mm glass-bottom dishes (Nest Biotechnology Co., LTD, USA) with 2% FBS for 24 h. Firstly, the cells were inoculated with RSV or His6 RSV at 4 ^{o}C for 30 min, and washed with ice-cold PBS to remove unbound viruses. Then 4 nM QD-NTA-Ni^{2+} were added to cells and incubated at 4 ^{o}C for 30 min. After that, the cells were fixed with 4% paraformaldehyde, and incubated with 2% BSA for 1 h. Cells were then stained with mouse monoclonal antibody against RSV phosphoprotein P (Abcam, UK) for 1.5 h at 37 ^{o}C and DyLight 488-conjugated goat anti-mouse IgG (Thermo Scientific, USA) for 1 h at 37 ^{o}C. Immunofluorescence images were captured with Olympus IX-81 inverted microscope equipped with Olympus IX2-DSU confocal scanning system and Rolera-MGi

EMCCD. Colocalization analysis were performed with Image-Pro Plus software (MedicaCybernetics, Inc.).

Live cell fluorescence imaging. HEp-2 cells, cultured in 35 mm glass-bottom dishes, were washed twice with ice-cold PBS, and His6 RSV was added to cells at 4 ^{o}C. After 30 min of incubation, the unbound viruses were removed and 4 nM QD-NTA-Ni^{2+} were added to cells at 4 ^{o}C for 10 min. After washed for 3 times, the dish was placed on the stage-top incubator (Tokai Hit Co., LTD, Japan) combined with Olympus IX-81 inverted microscope and shifted from 4 ^{o}C to 37 ^{o}C to initiate the entry of virus. Image series were recorded at frame interval of 14.2 s or 2 min. Moving trajectories of virus were analyzed with Image-Pro Plus software.

1. P. P. Hu, L. Q. Chen, C. Liu, S. J. Zhen, S. J. Xiao, L. Peng, Y. F. Li and C. Z. Huang, *Chem. Commun.*, 2010, **46**, 8285–8287.

2. Additional Figures

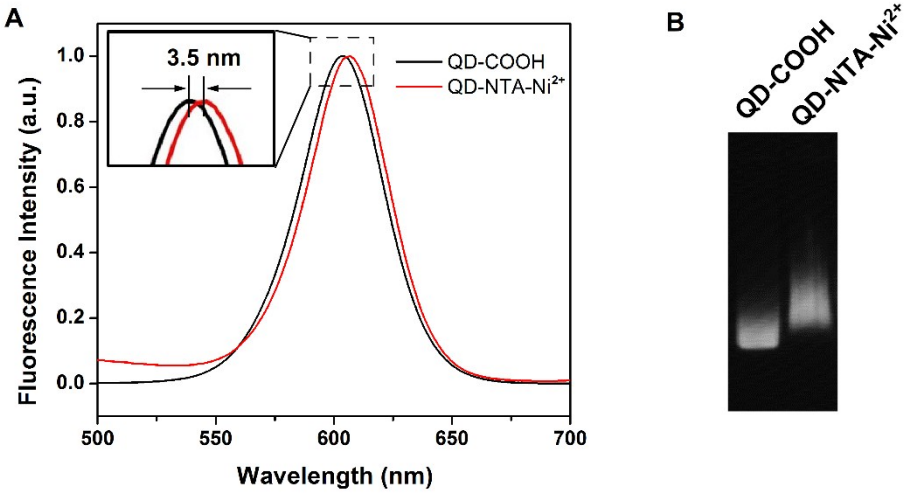

Fig. S1 Characterization of prepared QD-NTA-Ni^{2+}. (A) Fluorescence spectra of QD-COOH (black) and QD-NTA-Ni^{2+} (red) by applying an excitation wavelength at 370 nm. The red shift (3.5 nm) after modification of fluorescence peak position was shown in the inset. (B) Agarose gel electrophoresis of QD-COOH and QD-NTA-Ni^{2+}.

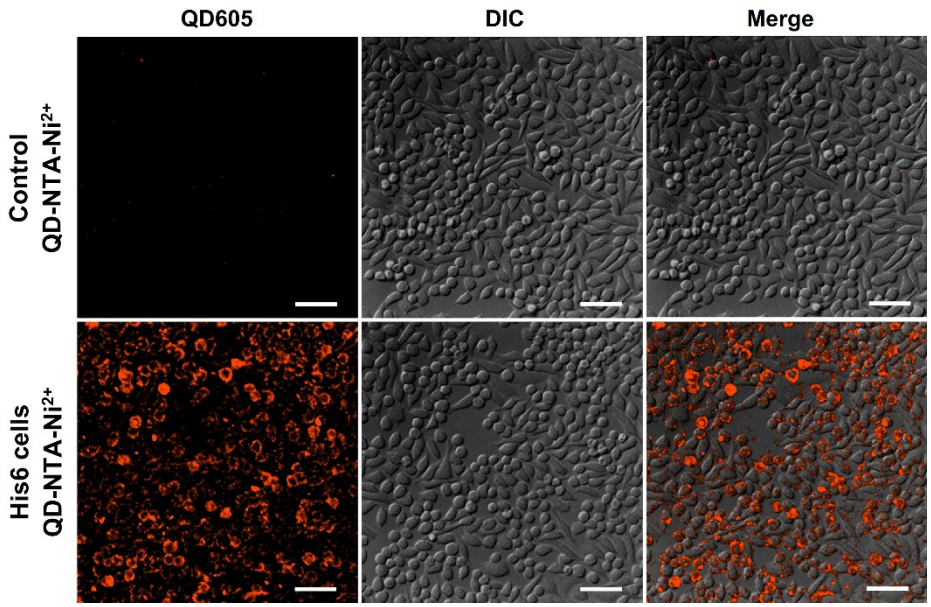

Fig. S2 Characterization of His tag modified HEp-2 cells by fluorescence microscopic imaging. HEp-2 cells were first modified with activated His6 for 30 min at room temperature, then incubated with QD-NTA-Ni^{2+} for 30 min at 4 °C. Scale bar, 20 μm.

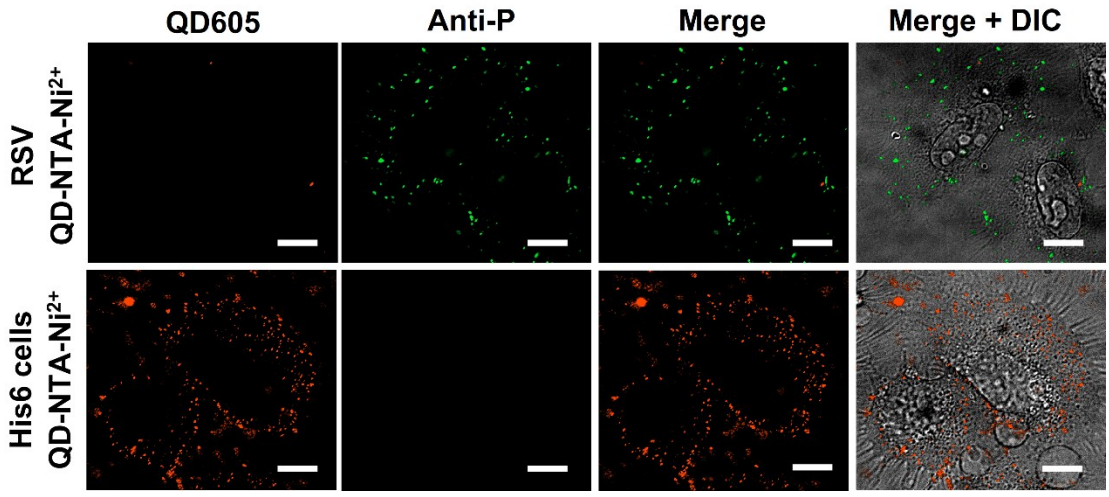

Fig. S3 Immunofluorescence imaging of RSV infected cells and QD-NTA-Ni^{2+} modified cells. Cells were inoculated with RSV at 4 °C for 30 min or His-tag activated His6 peptide at room temperature for 30 min, then incubated with QD-NTA-Ni^{2+} at 4 °C for 30 min. After being fixed, the cells were stained with mouse monoclonal antibody against P protein and DyLight 488-conjugated goat anti-mouse IgG (green). Scale bar, 10 μm.

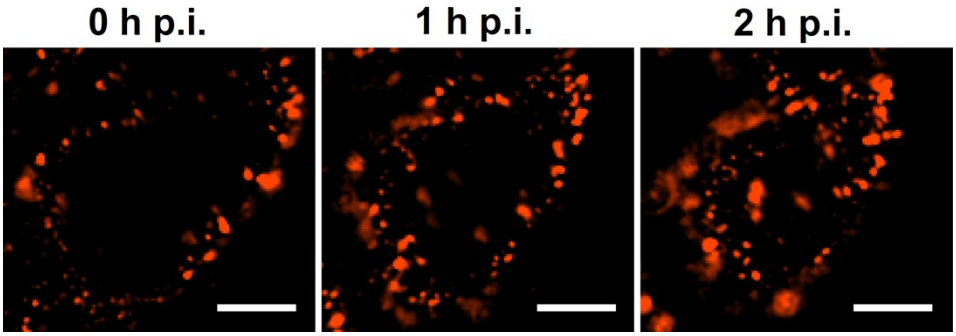

Fig. S4 Fluorescence imaging of QD labelled RSV infection cells at 0 h, 1 h and 2 h. Scale bar, 10 μm.

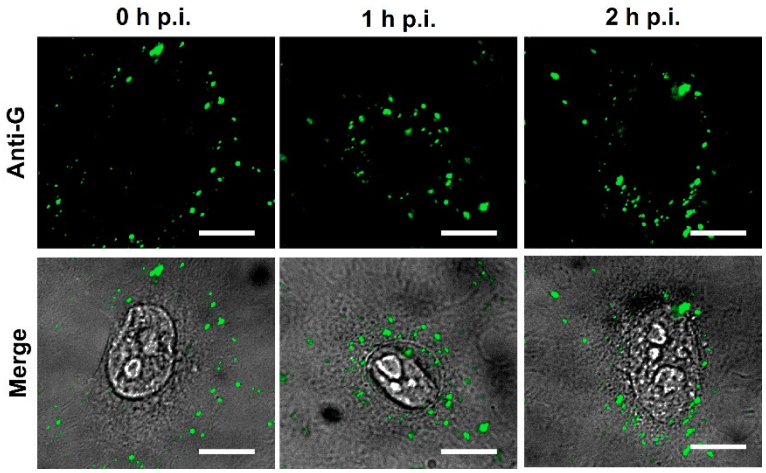

Fig. S5 Immunofluorescence imaging of RSV infected cells at 0 h, 1 h and 2 h post-infection.

Movie S1. Intracellular trafficking of QDs-labelled RSV in HEp-2 cells.