Supporting Information

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SI Text

Examination of Virus Binding by Confocal Immunofluorescence Microscopy. CHO k1 and CHO 15B cells were grown to near-confluency on glass coverslips in 24-well plates. Cells were washed twice with PBS containing 1 mM $CaCl_2$ and 0.5 mM Mg Cl_2 (PBS/Ca/Mg) and incubated with influenza A virus (IAV; strain WSN) at a multiplicity of infection of 2 (as determined by $TCID_{50}$) for 2 h at 20 °C. Endocytosis does not occur at this temperature. Incubations with IAV were performed in PBS/Ca/Mg or PBS/Ca/Mg containing 10% (vol/vol) FCS. Cells were washed twice with PBS/Ca/Mg and then fixed and permeabilized with 100% methanol. After blocking for 15 min with 10% normal goat serum, the cells were incubated for 1 h with a monoclonal antibody directed against the IAV nucleoprotein (HB-65; kindly provided by Ben Peeters, Central Veterinary Institute, Lelystad, Netherlands). After washing, the cells were incubated with a 1:400 dilution of Alexa Fluor 488 goat anti-mouse IgG (Molecular Probes) secondary antibody for 1 h. Nuclei were subsequently stained with DAPI, and after three washing steps, the coverslips were mounted in FluorSave (Calbiochem). Immunofluorescence staining was analyzed using a confocal laser-scanning microscope (Leica SPE-II).

Determination of Effects of Dynasore and 5-(*N*-ethyl-*N*-isopropyl) amiloride on Postentry Events and Cell Viability. CHO k1 cells were grown in 96-well plates and incubated for 3 h in the presence of 80 μM dynasore (Sigma-Aldrich), 80 μM 5-(*N*-ethyl-*N*-isopropyl)amiloride (EIPA; Sigma-Aldrich), or 80 μM dynasore plus 80 μM EIPA. Cell viability was determined by a Wst-1 cell viability assay (Roche) in accordance with the manufacturer's instructions. Both inhibitors also were examined at 80 μM concentrations for postentry effects on IAV infection. The entry assay was performed as described in *Experimental Procedures*. To determine the postentry effects of both inhibitors, CHO k1 and CHO 15B cells were inoculated with IAV WSN-Ren for 2 h in PBS containing 10% FCS in the absence of inhibitors. The inhibitors were subsequently added to the BafA1-containing culture medium, and were replaced after 3 h by culture medium containing only BafA1. For comparison, effects on entry were examined as well. Luciferase activity was determined as described in *Experimental Procedures*.

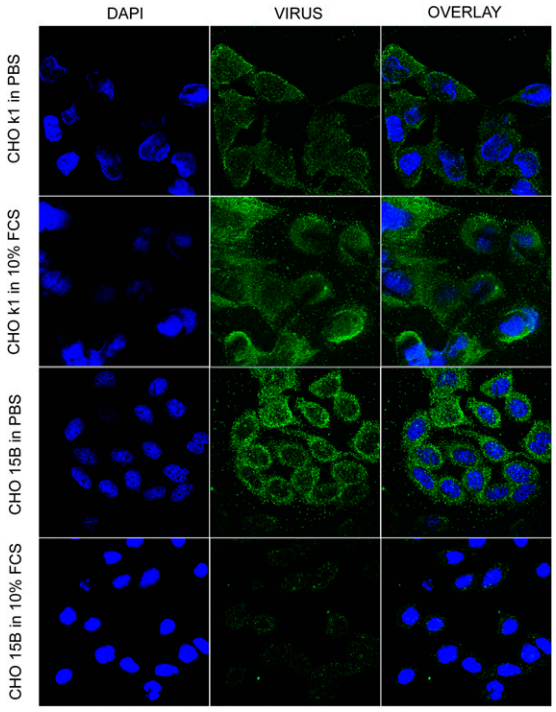

Fig. S1. Effect of serum on IAV (strain WSN) binding to CHO k1 and CHO 15B cells. Virus was bound to cells at 20 °C for 2 h in the presence or absence of 10% serum as indicated. Nuclei were stained with DAPI (blue), and virus was stained using an anti-nucleoprotein monoclonal antibody (green).

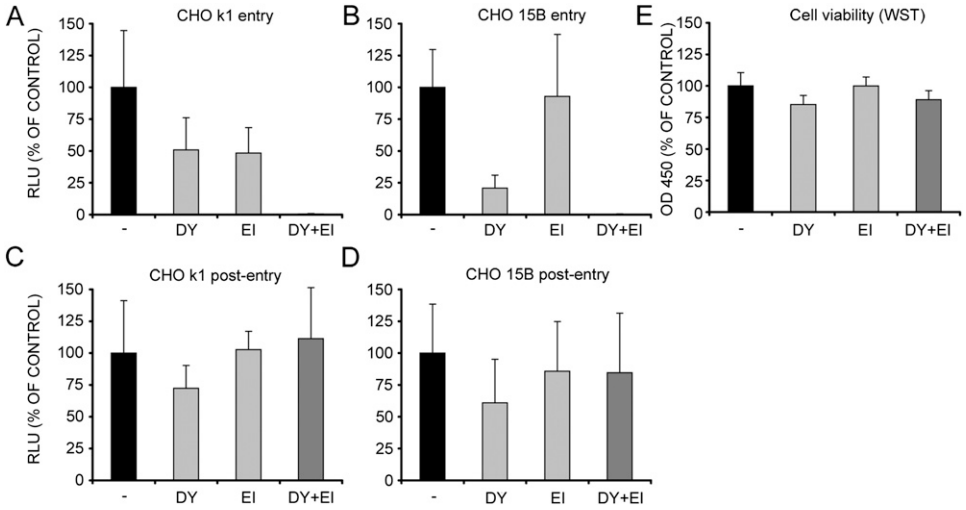

Fig. S2. The effect of dynasore and EIPA on postentry events and cell viability. Dynasore (DY) and EIPA (EI) were used at 80 μM. (*A*–*D*) Luciferase activity plotted relative to the controls with no inhibitors added (–). Inhibitors where present during entry of IAV (*A* and *B*) or postentry (*C* and *D*) using CHO k1 cells (*A* and *C*) or CHO 15B cells (*B* and *D*). (*E*) Cell viability of CHO k1 cells after incubation with the indicated inhibitors plotted relative to the control (–).