Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

Distal renal tubular system-on-a-chip for studying the pathogenesis of influenza A virus-induced kidney injury

Yueyue Huangfu,[a] Ji Wang,[a] Jiao Feng [a] and Zhi-Ling Zhang *[a]

[a] College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, P.R. China

* Corresponding author: College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, China.

E-mail address: zlzhang@whu.edu.cn (Z.-L. Zhang).

Materials and methods

Cytocompatibility and confinement evaluation of dRTSC

Cell growth status and channel confinement of dRTSC were observed at 24 h intervals using microscopy. On day 11, cells were labeled with Calcein-AM (Thermo Fisher Scientific, USA) and Propidium Iodide (PI, Sigma Aldrich, USA) to observe the viability.

Screening of the cell inoculation density

MDCK.2 cell suspensions of various concentrations (5×10^5, 1×10^6, 5×10^6, and 1×10^7 cells mL^{-1}) were inoculated into the dRTSC. Cell nuclei and membranes were labeled after 3 days of culture, and the

cell monolayers were observed by confocal microscopy. 1×10^6 cells mL^{-1} was chosen to inoculate finally.

Fluid shear force application

The fluid shear force was calculated approximated by the equation $\tau = 6\mu Q/wh^2$, where μ is the medium viscosity at 37°C (kg m^{-1} s), Q is the flow rate (m^3 s^{-1}), and w and h are the width (m) and height (m) of the channel, respectively. [1] The fluid shear force was chosen to resemble physiological conditions as closely as possible under the experimental conditions. After the cells had grown into continuous monolayers, a fluid shear force of 0.01 Pa was applied to the distal renal tubule and 0.05 Pa to the blood vessel. [2]

H1N1 Virus replication

The air chambers were drawn with a pencil on the 9-day SPF chicken embryos. The virus stock (H1N1, strain A/Puerto Rico/8/34) with a hemagglutination potency of 2^8 was diluted 20-fold with PBS and 1% penicillin and streptomycin to prepare a viral master mix, which was filtered through a 0.22 μm filter membrane and stored overnight at 4℃. A syringe was used to inject 200 μL of viral master mix into the allantoic cavity of each chick embryo, and the needle hole was sealed with paraffin. Chicken embryos were incubated at 37°C for

48 h and transferred to a 4°C refrigerator overnight. Allantoic fluid from chicken embryos was collected and centrifuged twice at 7000 rpm for 30 min to remove hemoproteins. Subsequently, the virus was concentrated by ultra-isolation, purified by ultra-isolation in a 15% to 60% sucrose gradient, and ultra-isolated again to remove sucrose and collect the virus. The acquired virus was dosed and stored in a -80°C refrigerator.

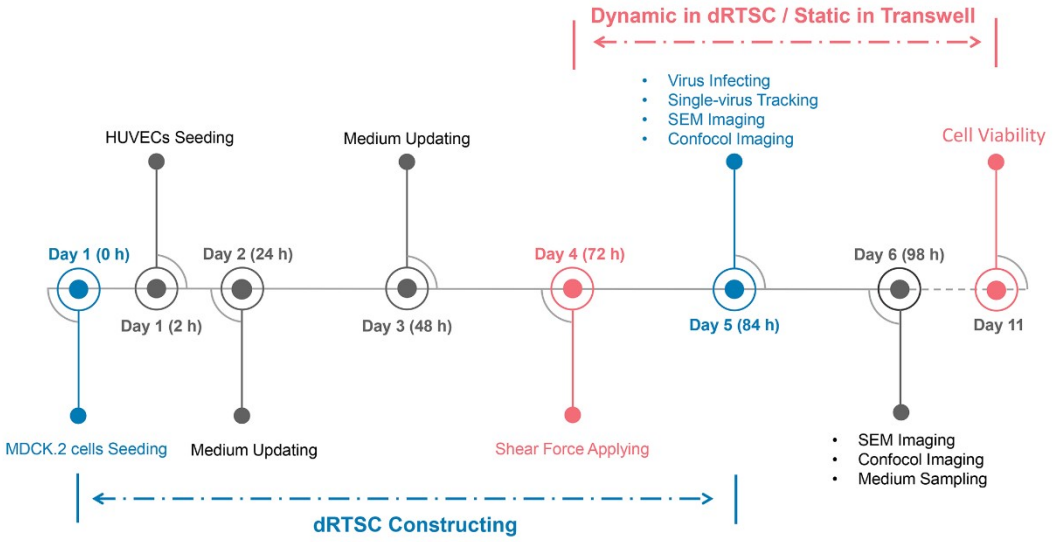

Fig. S1 Experimental procedure timeline. MDCK.2 cells were seeded in the upper channel and adhered to the porous membrane after 2 h. HUVECs were seeded in the lower channel and the chip was inverted until day 2. After the attachment of the two cell types, the medium was renewed to remove the unsuccessful cells, and the culture was continued until day 4. Shear force was applied in dRTSC while the static culture was maintained in Transwell for 12 h, cell polarization was characterized, and virus infection was induced. Cell polarization was recharacterized and the medium was sampled for Na element reabsorption analysis after 14 h of virus infection.

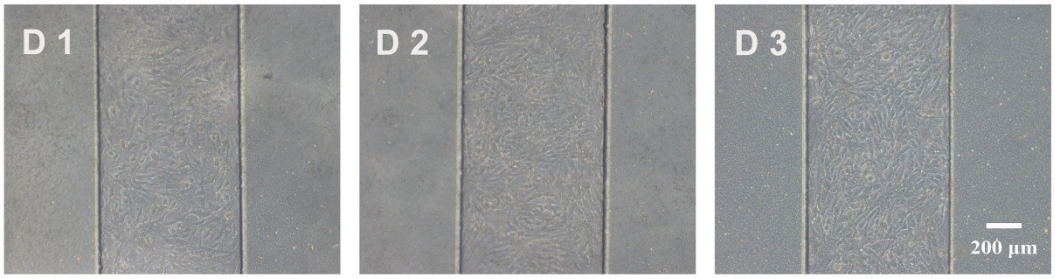

Fig. S2 The chip channel is confined and no cells grow outside the channel.

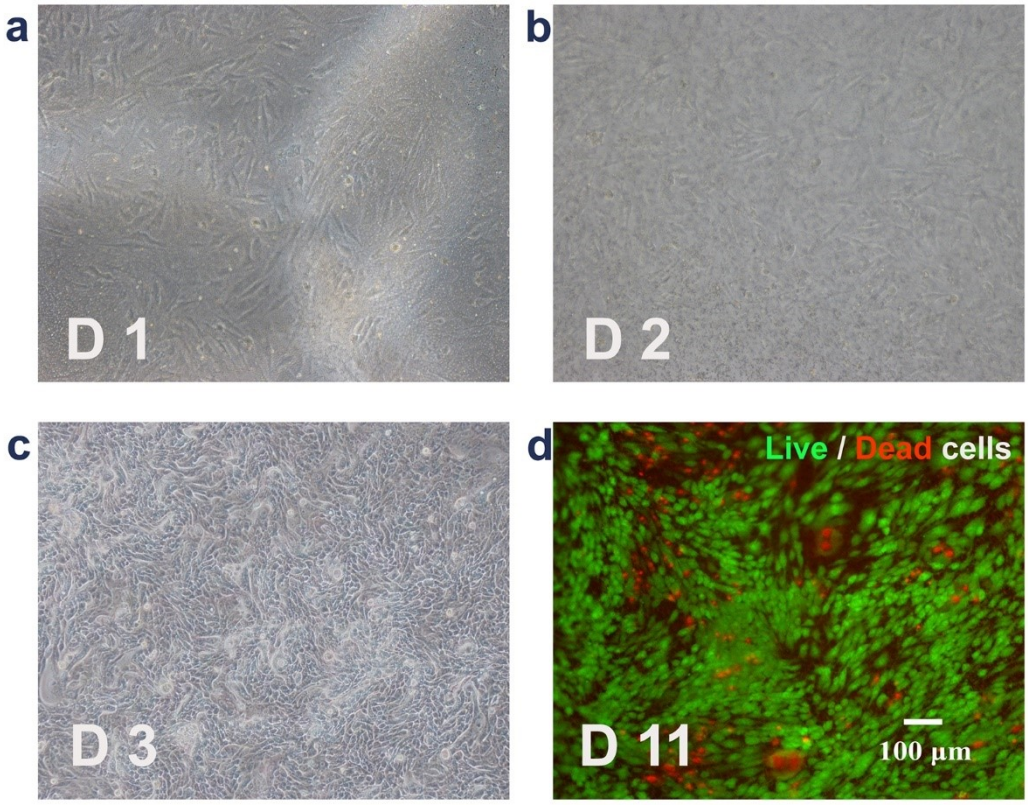

Fig. S3 Cell growth of dRTSC. a-c) Cells grew into a continuous monolayer after 3 days of culturing in dRTSC. d) On day 11, the number of cell deaths was at a low level.

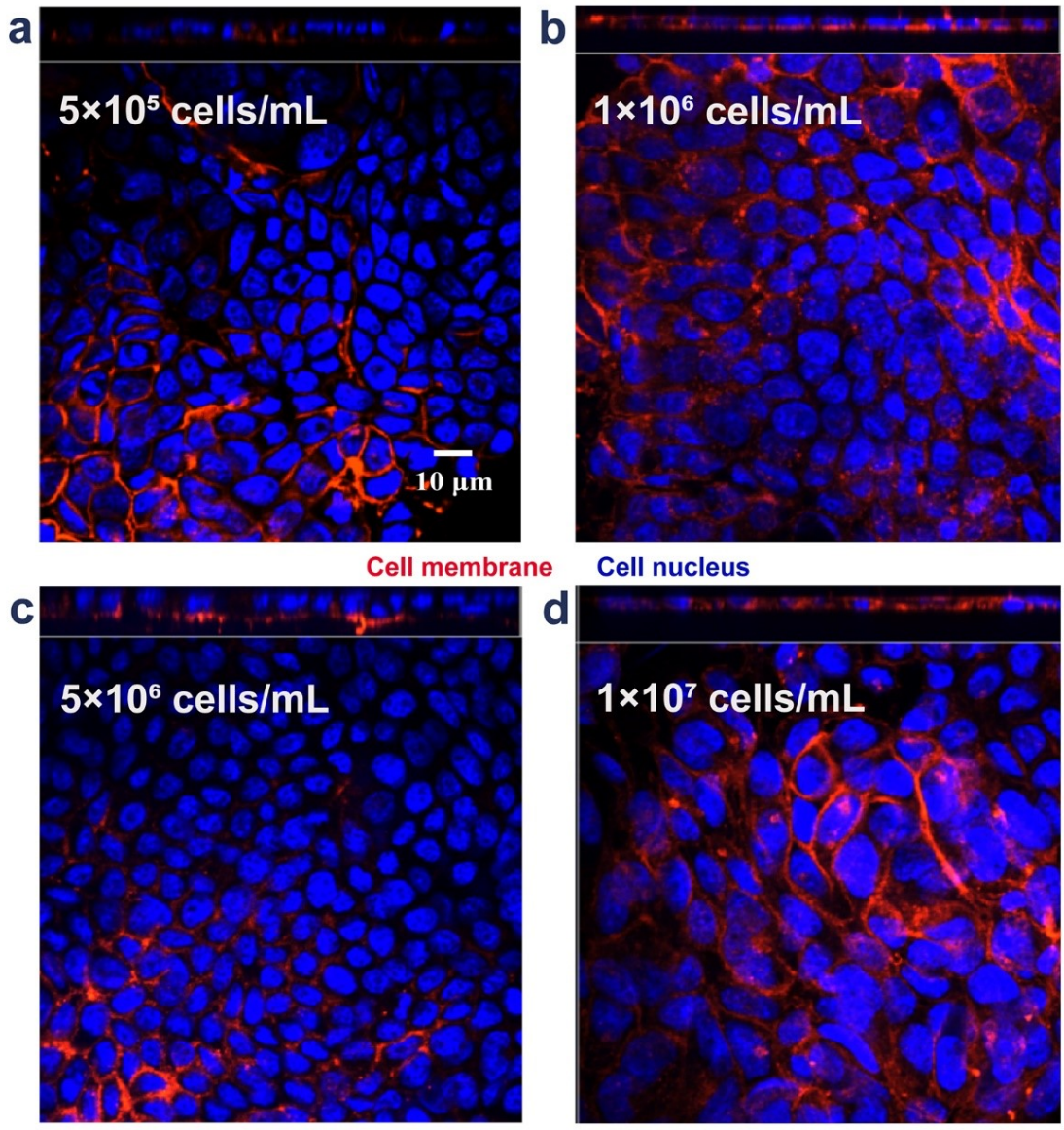

Fig. S4 Cell growth after 3 days of culturing in dRTSC at different cell densities.

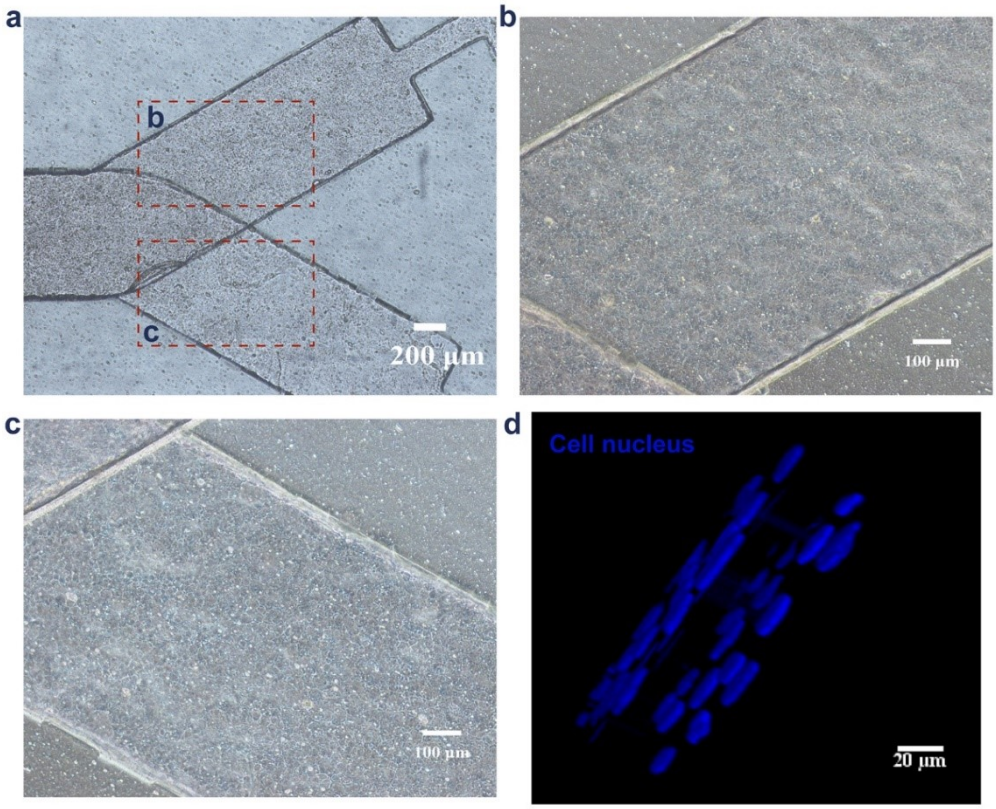

Fig. S5 The successful construction of dRTSC. a-c) Cell growth in both channels under bright field. d) Three-dimensional reconstruction of bilayer cell nuclei under fluorescence field.

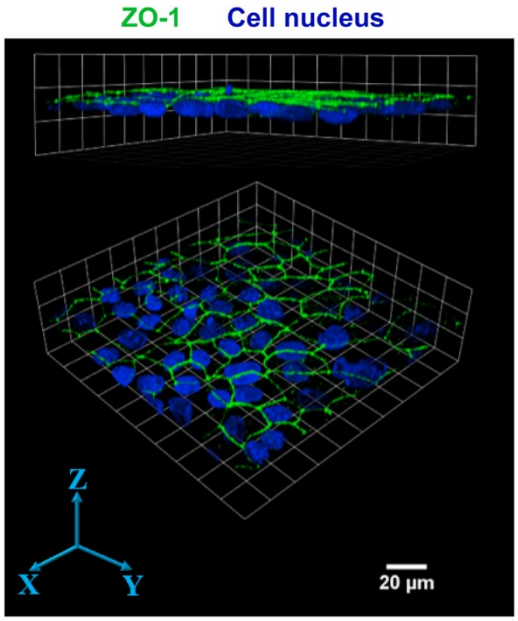

Fig. S6 Three-dimensional reconstruction of ZO-1 protein in MDCK.2 cells after application of fluid, with ZO-1 protein positioned between cells near the apical membrane.

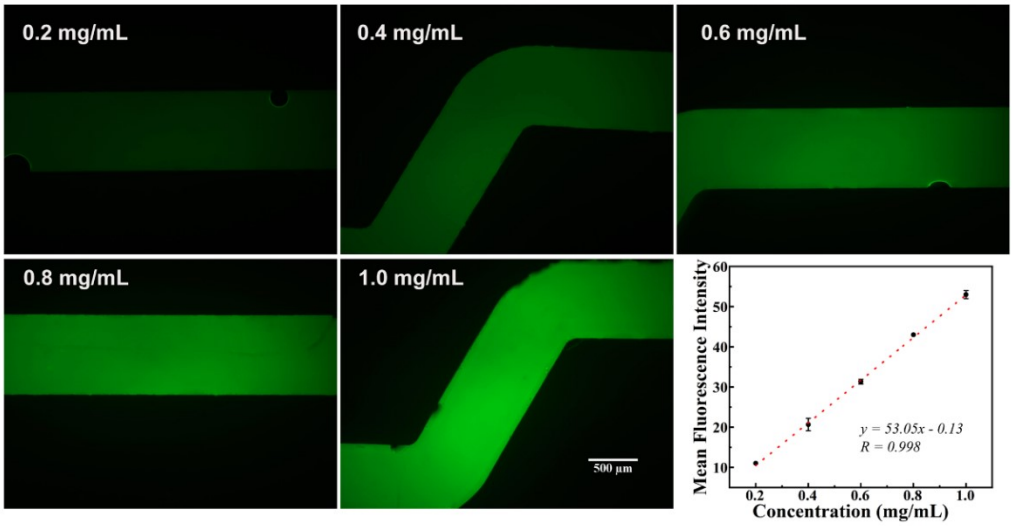

Fig. S7 A linear relationship between FITC-Dextran concentration and fluorescence intensity (0.2-1.0 mg mL^{-1}).

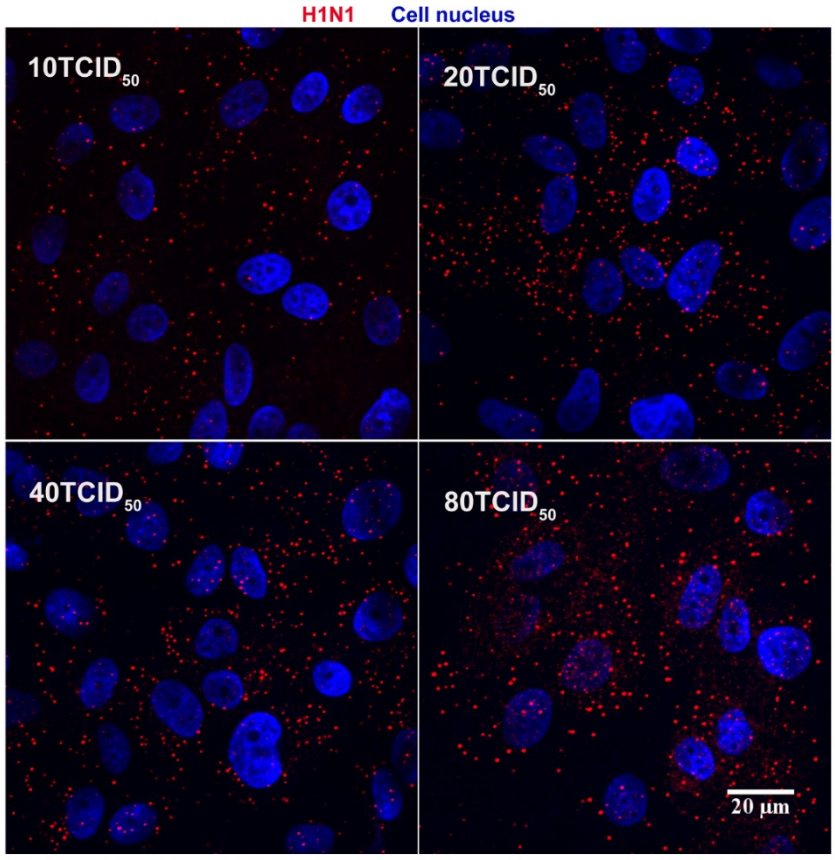

Fig. S8 Infection of MDCK.2 cells with H1N1 virus at different $TCID_{50}$.

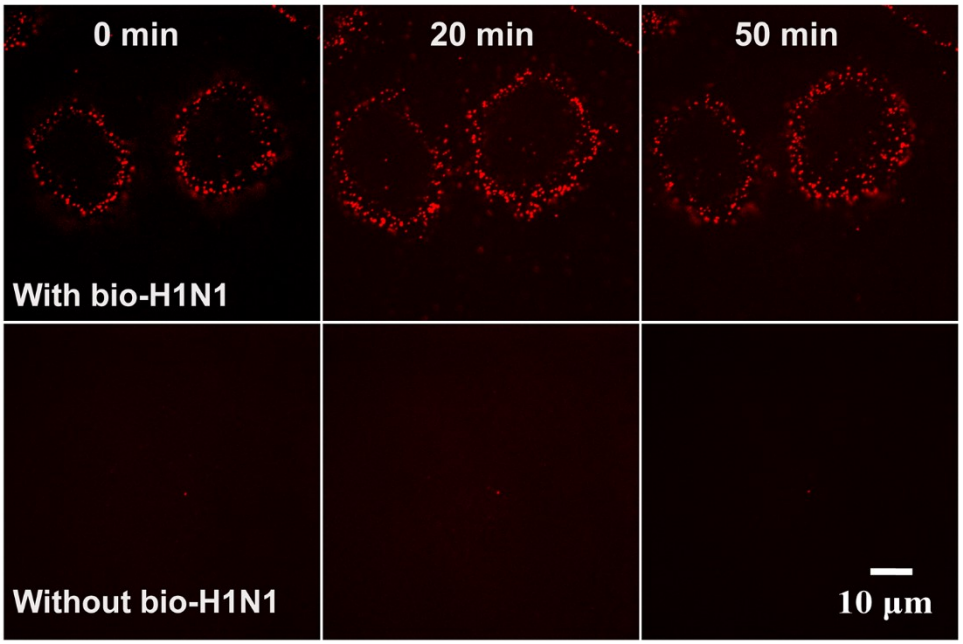

Fig. S9 Successful labeling and time series of H1N1 virus envelope proteins.

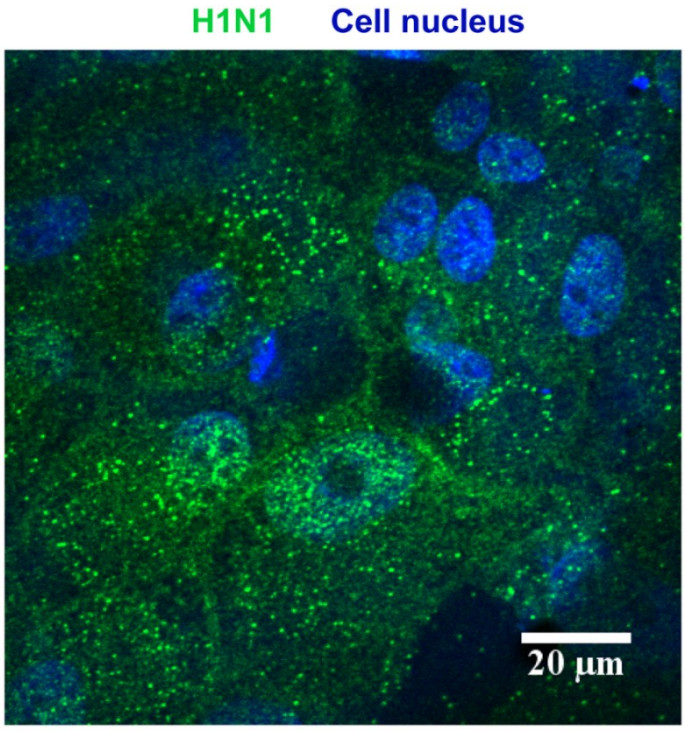

Fig. S10 Immunofluorescence results of H1N1 virus infecting MDCK.2 from the basement membrane side.

Movie S1. Cy3-labeled H1N1 virus tracking in blood vessel channel of dRTSC.Movie S1. Cy3-H1N1 Virus Tracking.mp4

Publication license for Fig. 1a Publication License for Fig. 1a.pdf

Publication license for Fig. 6a Publication License for Fig. 6a.pdf

1 K. J. Jang, A. P. Mehr, G. A. Hamilton, L. A. McPartlin, S. Chung, K. Y. Suh and D. E. Ingber, *Integr. Biol.*, 2013, **5**, 1119-1129.

2 S. Weinbaum, Y. Duan, L. M. Satlin, T. Wang and A. M. Weinstein, *Am J Physiol Renal Physiol*, 2010, **299**, F1220-1236.