Supplementary information, Data S1 Materials and Methods

Virus and cells

The influenza virus used in this study was H5N1 (A/Jilin/9/2004(H5N1)). Live virus experiments were performed in Biosafety Level 3 facilities under governmental and institutional guidelines. Viruses were propagated by inoculation into 9- to 11-day-old SPF embryonated fowl eggs via the allantoic route. Hemagglutinating allantoic fluid was collected from eggs and used directly.

The human lung adenocarcinoma A549 cell line was purchased from ATCC and cultured in F-12/Ham (Gibco) medium supplemented with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37 °C under a 5% carbon dioxide atmosphere. MDCK cells were purchased from the Peking Union Medical College Cell Culture Center and cultured in DMEM (Gibco) medium supplemented with 10% FBS, 100 U/mL penicillin and 100 μg/ml streptomycin at 37 °C under a 5% carbon dioxide atmosphere.

Reagents

For these experiments, 3-methyladenine (3-MA) and chloroquine diphosphate salt (CQ) were purchased from Sigma-Aldrich. The rabbit anti-LC3 monoclonal antibody and mouse anti-beta-actin monoclonal antibody were purchased from Sigma-Aldrich. The primers were all synthesized by Invitrogen.

Virus titration

Ten fold dilutions of the virus were utilized to inoculate MDCK cells in a 96-well plate, and infected cells were maintained in culture for 72 hours. Viral titers were calculated using the Reed-Muench method and expressed as $TCID_{50}$ per milliliter of supernatant.

Cell viability assay:

A549 cells were treated with virus titrated to 4 MOI or an equal volume of vehicle for

48 hours. The cell viability was then determined by an MTS assay (Promega). In the drug rescue assays, 3-MA (3 mM) or CQ (3, 10, 30 μM) was added one hour before or CQ (30 μM) were added 1, 3, 6 hours after the viral administration.

Animal handling

Animal experiments were conducted in the animal facility at the Institute of Basic Medical Sciences, Peking Union Medical College, and the Institute of Military Veterinary Medicine, Academy of Military Medical Sciences, in accordance with governmental and institutional guidelines. The animal experiments were approved by the IACUC of the Institute of Military Veterinary Medicine, Academy of Military Medical Sciences and the approved number was 2012-021. Four-week-old BALB/c mice were purchased from the Institute of Laboratory Animal Science, PUMC, Beijing and the Vital River Laboratories. All mice were caged in a specific pathogen-free facility in groups of five or less and fed a laboratory autoclavable rodent diet *adlibitum*. Lung injury was induced in mice through intratracheal injection of vehicle control or virus (10^6TCID$_{50}$) using a technology that we have previously described for SARS-Spike protein and acid-induced lung injury (Kuba, K.*, et al.Nat Med***11**, 875-879 (2005)). In the therapeutic treatment group, CQ (50 mg/kg) was injected intraperitoneally 6 hours and once per day for 1 week after vehicle control or H5N1 virus instillation; in the prophylactic treatment group, CQ (50 mg/kg) was injected intraperitoneally 2 hours and 0.5 hour before vehicle control or H5N1 virus instillation.

Lung tissue slides for histopathological examination

Four or five days after the instillation of either vehicle control or virus, the mice were sacrificed.The lungs were dislodged from the thoracic cavity and placed in a glass vial containing approximately 50 ml of fixative. Each glass vial was assigned a number unknown (with respect to treatment) to the pathologists. The lungs were fixed for at least 48 h before further processing. The formalin-fixed mouse lungs were embedded in paraffin, thin-sectioned coronally, and mounted on glass microscope slides using

standard histopathological techniques. The sections were stained with hematoxylin–eosin. For each group, at least three mice were sacrificed and mice lung tissues were examed under 200× and 400× objective. The number of inflammatory cells was counted and analyzed from at least 100 visual fields under 200× objective from 8-10 section slides of each mouse lung tissue. The inflammatory cells were primarily monocytes and neutrophils, monocytes were recognized by their relatively large, oval, reniform or hippocrepiform nucleus and neutrophils were recognized by their 3-5 lobulated nucleus.

Assessment of pulmonary edema

Mice were treated as described above. Lungs were assessed for their wet weight four or five days after virus injection and dried in a 65 °C oven for 48 hours to obtain the dry weight.

Survival rate and body weight changes

Mice were treated as described above. The survival rate and changes of body weight of each group (n=10) were recorded consecutively for 8 days. Survival rates were analyzed by Kaplan-Meier survival analysis and changes in body weight were analyzed by Two-way ANOVA and a two-tailed t-test.

Western Blot Analysis

Mice were intratracheal instillated of H5N1 virus (10^6 $TCID_{50}$) and the lung tissue were collected at the indicated time points. Lung tissues were homogenized in ice-cold lysis buffer [50 mM tris-HCl (pH 7.5), 150 mM NaCl, 1.0% Triton X-100, 20 mM EDTA, 1 mM Na3VO4, 1 mM NaF, and protease inhibitors]. Tissue lysates were resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and proteins were transferred onto a nitrocellulose filter membrane. Membranes were incubated with the

appropriate primary antibodies (LC3 and beta-actin were purchased from Sigma) and then with HRP-conjugated secondary antibodies. Binding of secondary antibody was visualized with the Kodak film exposure detection system, and the film was scanned and analyzed.

Real-time quantitative PCR analysis

Mice were intratracheal instillated of H5N1 virus (10^6 $TCID_{50}$) and the lung tissue were collected at the indicated time points. Total RNA were isolated with Trizol reagent (Invitrogen). Complementary DNA (cDNA) was synthesized from 1.0μg of total RNA with the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). PCR amplification assays were performed with the FastStart Universal SYBR Green Master mix with ROX (Roche) on an ABI 7500 Real-time PCR System (Applied Biosystems). Samples were normalized based on the expression the gene encoding mice beta-actin as a reference. The specific primers used were as follows: M1 forward: 5'-CTCTCTATCATCCCGTCAG-3'; M1 reverse: 5'-GTCTTGTCTTTAGCCATTCC-3'; M2 forward: 5'-ATTGTGGATTCTTGATCGTC-3'; M2 reverse: 5'-TGACAAAATGACCATCGTC-3'; IL-1β forward: 5'-GGACAGAATATCAACCAACAA-3'; IL-1β reverse: 5'-TTACACAGGACAGGTATAGATT-3'; IL-6 forward: 5'-CCGCTATGAAGTTCCTCT-3'; IL-6 reverse: 5'-CTCTGTGAAGTCTCCTCTC-3'; TNF-α forward: 5'-TCTCAGCCTCTTCTCATTC-3'; TNF-α reverse:

5'-GCCATTTGGGAACTTCTC-3'; β-actin forward: CTCTCCCTCACGCCATCC，

β-actin reverse: CGCACGATTTCCCTCTCAG.

Statistical analyses

All data are shown as means ± SEM. Measurements at single time points were analyzed by ANOVA and, if they demonstrated significance, the measurements were further analyzed by a two-tailed t test. Survival data were analyzed by Kaplan-Meier survival analysis. All statistical tests were conducted using GraphPad Prism 5.0 (GraphPad Software, San Diego, CA, USA). Findings were considered statistically significant for $p< 0.05$.