Supplementary Information for

High ambient temperature dampens adaptive immune responses to influenza A virus infection

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Supplementary Materials and Methods

Antibiotic treatment.

Mice were treated for 4 wks with ampicillin (1 g/L; Nacalai Tesque), vancomycin (500 mg/L; Duchefa Biochemie), neomycin sulfate (1 g/L; Nacalai Tesque), metronidazole (1 g/L; Nacalai Tesque), gentamicin (10 mg/L; Nacalai Tesque), penicillin (100 U/ml; Nacalai Tesque), streptomycin (100 U/ml; Nacalai Tesque), and amphotericin B (0.25 mg/L; Nacalai Tesque) in drinking water as previously described (1, 2). Antibiotic-containing water was changed twice a wk.

Virus infection *in vivo*.

Recombinant influenza A virus expressing the MHC-I OVA peptide SIINFEKL (PR8-OT-I) in the NA stalk of the A/PR8 backbone was generated through reverse genetics using specific primers (forward: 5'- GTT GAT TAT ACT ACT TCC AGT TTG AAT TGA ATG -3'; reverse: 5'- TTT GAA AAA CTG ACC TAT AAA AAT AGC ACC TG -3') (3). WT A/PR8 and recombinant PR8-OT-I viruses were grown in the allantoic cavities of 10-d-old fertile chicken eggs at 35 °C for 2 d. Viral titer was quantified by a standard plaque assay using Madin-Darby canine kidney (MDCK) cells and viral stock was stored at −80 °C. For i.n. infection, mice were fully anesthetized by i.p. injection of pentobarbital sodium (Somnopentyl, Kyoritsu Seiyaku Co., Ltd., Tokyo, Japan) and then infected by i.n. application of 30 μL of virus suspension (30–1,000 pfu of influenza virus in PBS). This procedure leads to upper and lower respiratory tract infection (4).

ZIKV (ATCC VR-84) and SFTSV (a gift from S. Morikawa, National Institute of Infectious Diseases, Tokyo, Japan) were amplified on Vero cells and stored at −80 °C until use. The infectious titer was determined by a standard plaque assay or a focus-forming assay using Vero cells, as described previously (5). Mice were infected i.p. with 6×10^5 pfu of ZIKV or 6×10^6 $TCID_{50}$ of SFTSV. All experiments with SFTSV were performed in enhanced biosafety level 3 (BSL-3) containment laboratories at the University of Tokyo, in accordance with the institutional biosafety operating procedures.

Flow cytometry.

The single-cell suspensions of lung samples were prepared as previously described (4). For tetramer staining, cells were incubated with PE-labeled tetramer specific for H2-D^b complexed with peptides from the viral nucleoprotein (NP) in 0.1 mL of 1% FBS in PBS for 30 min on ice. After washing, samples were resuspended in 1%

paraformaldehyde in PBS. For LC3B staining, cells were fixed and permeabilized using a Cytofix/Cytoperm kit (BD Biosciences), and intracellularly stained with rabbit polyclonal antibody against LC3B (#2775; Cell Signaling Technology) followed by Alexa Fluor 488 goat anti-rabbit IgG (Invitrogen). Flow cytometric analysis was performed with a FACSVerse flow cytometer (BD Biosciences). The final analysis and graphical output were performed using FlowJo software (Tree Star, Inc.).

CD4 and CD8 T-cell responses.

$CD4^+$ T cells and $CD8^+$ T cells were isolated from the spleen of mice infected with A/PR8 influenza virus, ZIKV, or SFTSV or immunized with inactivated influenza virus vaccine and aluminum adjuvant at 10 or 14 d post infection or vaccination using anti-CD4 microbeads or anti-CD8 microbeads (Miltenyi Biotec) according to the manufacturer's instructions. A total of 10^5 $CD4^+$ T cells and $CD8^+$ T cells were restimulated with 10^5 heat-inactivated virion-, influenza virus NP peptide (ASNENMETM, H-$2D^b$)-, or OVA peptide (SIINFEKL)-pulsed antigen presenting cells (APCs) for 72 h at 37 °C, respectively (1). IFN-γ production in supernatants was measured in triplicates by ELISA. For intracellular staining, a total of 10^5 $CD4^+$ T cells were cultured with 10^5 heat-inactivated virion-pulsed APCs for 6 h, and 10 μg/ml of brefeldin A (Nacalai Tesque) was added for an additional 12 h. Cells were then surface stained with anti-CD4-FITC and anti-CD44-PE (eBioscience) antibodies. Then, cells were fixed and permeabilized using a Cytofix/Cytoperm kit (BD Biosciences), and intracellular stained with APC-labeled anti-mouse IFN-γ antibody (clone XMG1.2; eBioscience) (4).

Measurement of virus titers and anti-A/PR8 antibodies.

Serum and bronchoalveolar (BAL) fluid were collected for measurement of virus titer and virus-specific IgG antibodies from the mice. The BALF was collected by washing the trachea and lungs twice by injecting a total of 2 ml PBS containing 0.1% bovine serum albumin (BSA). The virus titer was measured as follows: aliquots of 200 μl of serial 10-fold dilution of the BALF by PBS containing 0.1% BSA were inoculated into MDCK cells in 6-well plates. After 1 hour of incubation, each well was overlaid with 2 ml of agar medium. The number of plaques in each well was counted 2 d after inoculation (4).

The levels of IgG antibodies against A/PR8 influenza virus were determined by ELISA as described previously (6). Standards for A/PR8-, ZIKV-, and SFTSV-reactive IgG antibody titration were prepared from the serum of the virus-infected mice, and

expressed as the same arbitrary units (160-unit). The antibody titers of unknown specimens were determined from the standard regression curve constructed by twofold serial dilution of the 160-unit standard for each assay.

OT-I $CD8^+$ T cell proliferation.

Naïve OT-I $CD8^+$ T cells were isolated from the spleens of $CD45.1^+$ OT-I transgenic mice using a $CD8^+$ T cell isolation kit according to the manufacturer's instructions (>95% purity). Purified OT-I $CD8^+$ T cells were labeled with 5 μM carboxyfluoresceinsuccinimidyl ester (CFSE) (Invitrogen) for 10 min at 37 °C and washed three times in cold PBS. On the day before PR8-OT-I virus infection, 1×10^6 T cells were injected i.v. into cold-, RT-, or high heat-exposed C57BL/6 mice ($CD45.2^+$). The mLNs were collected at 5 d postinfection and single-cell suspensions were stained with anti-CD8α-APC and anti-CD45.1-eFluor 450. $CD45.1^+$ $CD8\alpha^+$ T cells were analyzed for proliferation by flow cytometry.

Isolation of lung and mLN DCs and restimulation of naïve OT-I $CD8^+$ T cells.

Cold-, RT-, or high heat-exposed mice were infected i.n. with 1,000 pfu of PR8-OT-I influenza virus. Three days later, $CD11c^+$ DCs were isolated from the mLNs of PR8-OT-I influenza virus-infected mice using anti-CD11c microbeads (Miltenyi Biotec). Naïve OT-I $CD8^+$ T cells were isolated from single-cell suspensions of OT-I TCR transgenic mice spleens using a $CD8^+$ T cell isolation kit (Miltenyi Biotec) according to the manufacturer's instructions (>95% purity). A total of 2×10^5 naïve OT-I $CD8^+$ T cells were restimulated with 1×10^5 DCs for 72 h at 37 °C. A total of 1×10^5 splenic $CD11c_+$ DCs isolated from mice infected with PR8-OT-I influenza virus were co-cultured with 2×10^5 naïve OT-I CD8 T cells as a negative control. IFN-γ production in the supernatant was measured in triplicate by ELISA.

In vivo tracking of antigen-captured respiratory DCs.

Mice were inoculated intranasally with 30 μg of DQ-ovalbumin (DQ-OVA) (D12053; Thermo Fisher Scientific). Six hours later, mice were infected with 1,000 pfu of PR8 viruses. Eighteen hours after infection, mLNs were collected and single-cell suspension were stained with anti-CD11c (HL3, BD Biosciences)-APC. DQ-OVA^+ $CD11c^+$ DCs were analyzed by flow cytometry.

Parabiosis.

Pairs of age matched underfed C57BL/6 (CD45.2) and ad libitum B6-Ly5.1 (CD45.1)

mice were surgically joined for parabiosis as described previously (7) at the time of infection. In brief, mice were fully anesthetized by i.p. injection of pentobarbital sodium (Somnopentyl, Kyoritsu Seiyaku Co., Ltd., Tokyo, Japan). After shaving the corresponding lateral aspects of each mouse, matching skin and fascia of anesthetized mice were longitudinally incised, and the pair of fascia and skin was joined.

Induction of autophagy *in vivo*.

To study the effects of autophagy in the induction of influenza virus-specific adaptive immune responses, food intake of RT-exposed mice kept 1 per cage was restricted to 2 g a day which is comparable to those of high heat-exposed group (Fig. S1B). These mice had free access to drinking water. Some groups were treated i.v. with rapamycin (LC Laboratories; 75 μg per kg body weight) daily from day -2 to day 9 during infection (8).

Immunoblot analysis.

The BALF was collected by washing the trachea and lung twice by injecting a total 2 ml of RIPA buffer (10 mM TRIS pH 7.4, 150 mM NaCl, 1% NP40, 1 mM EDTA, 0.1% sodium dodecyl sulphate [SDS], 0.1% sodium deoxycholate). Cell lysates were run on SDS-PAGE and blotted to PVDF membrane and developed with rabbit polyclonal antibody against LC3B (#2775; Cell Signaling Technology), followed by incubation with horseradish peroxidase-conjugated anti-rabbit IgG (Jackson Immuno Research Laboratories). The PVDF membranes were then treated with Chemi-Lumi One Super (Nacalai Tesque) to elicit chemiluminescent signals, which were detected and visualized using an LAS-4000 Mini apparatus (GE Healthcare).

Isolation of bacterial DNA.

Bacterial DNA was isolated as described previously (9). Briefly, stool samples harvested directly from the mouse cecum were homogenized in 500 μL of TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0). Glass beads, Tris-phenol buffer, and 10% SDS were added to the bacterial suspensions, and the mixtures were vortexed vigorously for 10 sec by using a FastPrep FP100 A (BIO 101). After incubation at 65°C for 10 min, the solutions were vortexed and incubated again at 65°C for 10 min. Bacterial DNA was then precipitated in isopropanol, pelleted by centrifugation, washed in 70% ethanol, and resuspended in TE buffer. SYBR Premix Ex *Taq* II (Takara) and a LightCycler (Roche Diagnostics) were used for quantitative PCR with the following primers: 16S rRNA forward, 5'-GTGCCAGCMGCCGCGGTAA-3', and reverse, 5'-

GGACTACHVGGGTWTCTAAT-3’ (10).

Sequencing of 16S rRNA gene.

Two step PCRs were performed for the purified DNA samples to obtain sequence libraries. The first PCR was performed to amplify using a 16S (V3-V4) Metagenomic Library Construction Kit for NGS (Takara Bio Inc., Kusatsu, Japan) with primer pairs of 341F (5’-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CCT ACG GGN GGC WGC AG-3’) and 806R (5’-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GGG ACT ACH VGG GTW TCT AAT-3’) corresponding to the V3-V4 region of the 16 rRNA gene. The second PCR was done to add the index sequences for Illumina sequencer with barcode sequence using the Nextera XT Index kit (Illumina, San Diego, CA). The prepared libraries were subjected to the sequencing of paired-end 250 bases using the MiSeq Reagent Kit v3 on the MiSeq (Illumina) at the Biomedical Center, Takara Bio. Processing of sequence data, including operational taxonomic unit (OTU) definition and taxonomy assignment was performed using CD-HIT-OTU 0.0.1 and QIIME ver. 1.8, respectively (11).

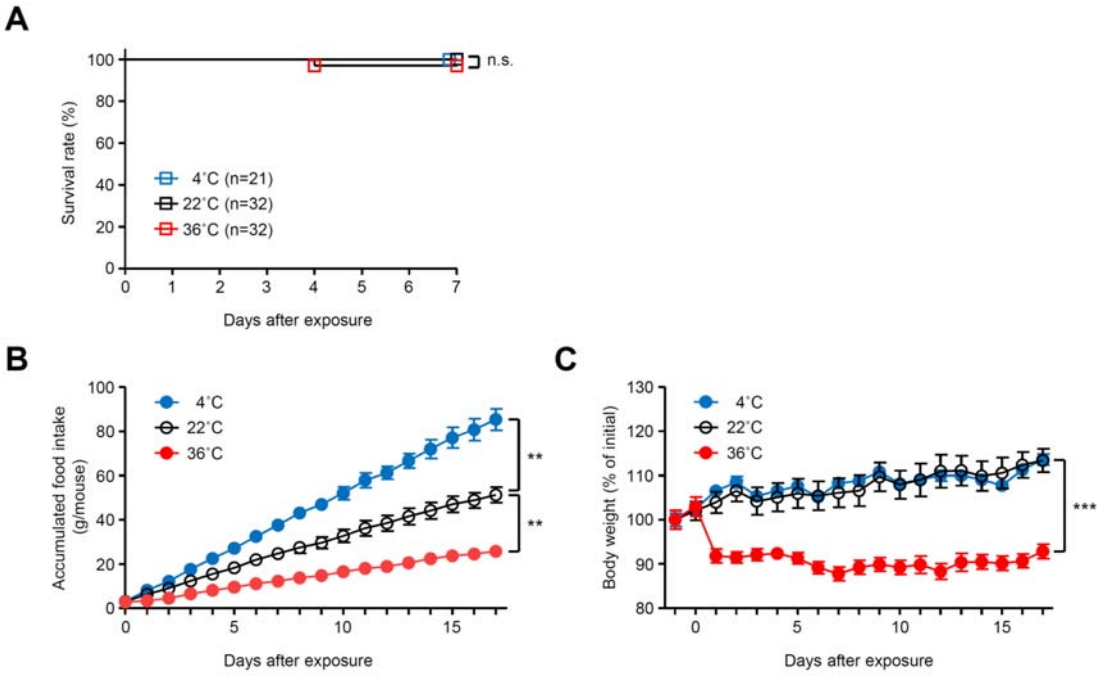

Figure S1. The effect of cold- or warm-exposure on survival, food intake, and body weight. Naïve mice were kept at 4, 22, or 36˚C. Survival (A), food intake (B) and body weight change (C) are depicted. ***P* <0.01, ****P* <0.001; n.s., not significant. Statistical significance was measured by log-rank (Mantel-Cox) test (A) or one-way ANOVA and Tukey's test (B and C).

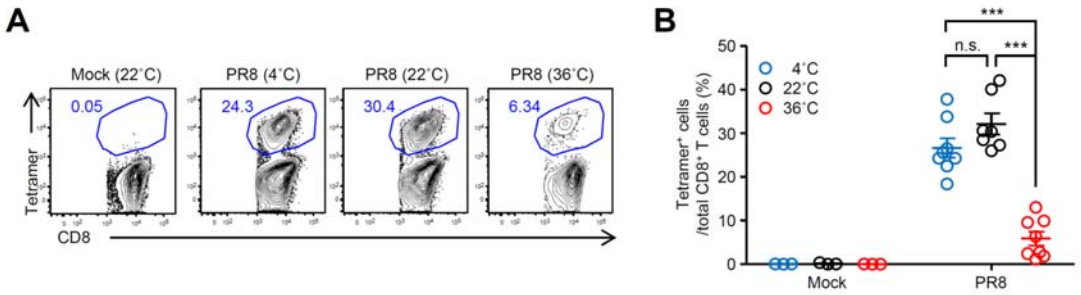

Figure S2. The effect of cold- or warm-exposure on induction of influenza virus-specific CD8$^+$ T cells. Mice were kept at 4, 22, or 36˚C for 7 d before influenza virus infection (30 pfu per mouse) and throughout infection. (**A**) Ten days later, lymphocytes were isolated from the lung. Influenza virus-specific CD8$^+$ T cells were then detected using the H-2D^b influenza virus NP tetramer. Plots are gated on total CD8$^+$ T cells and numbers indicate percent tetramer-positive CD8$^+$ T cells (**B**) Percentages of influenza virus-specific CD8$^+$ T cells in the lung are shown. ***$P < 0.001$; n.s., not significant (one-way ANOVA and Tukey's test).

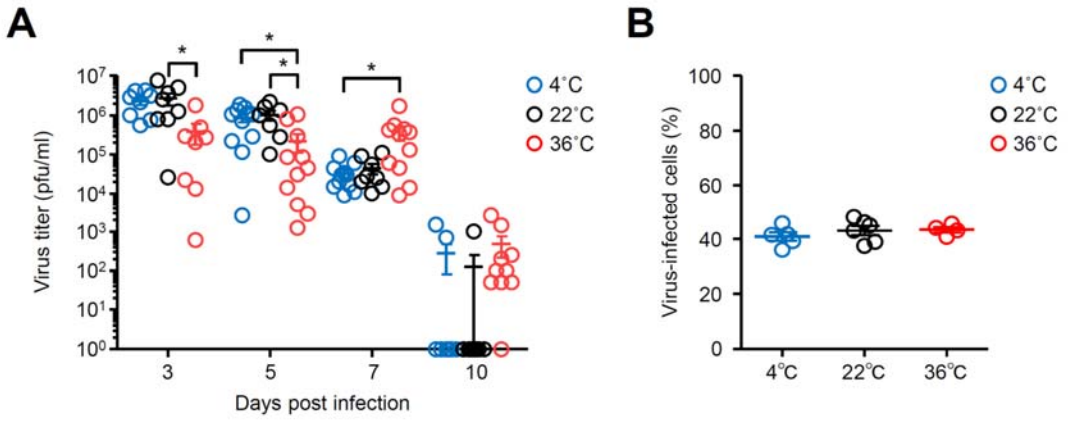

Figure S3. Influenza virus replication in the lung of cold-, RT-, or warm-exposed mice. Mice were kept at 4, 22, or 36˚C for 7 d before influenza virus infection (1,000 pfu per mouse) and throughout infection. (A) The BALF were collected at indicated time points and virus titers were measured by plaque assay. The data are pooled from three independent experiments. (B) The lung was collected from influenza virus-infected mice at 5 d post infection. The single-cell-suspensions of lung samples were stained with anti-influenza virus M2 protein and anti-CD45.2 antibodies. The ratio of influenza virus M2 protein-positive cells among CD45.2 negative cells are shown. *P <0.05 (one-way ANOVA and Tukey's test).

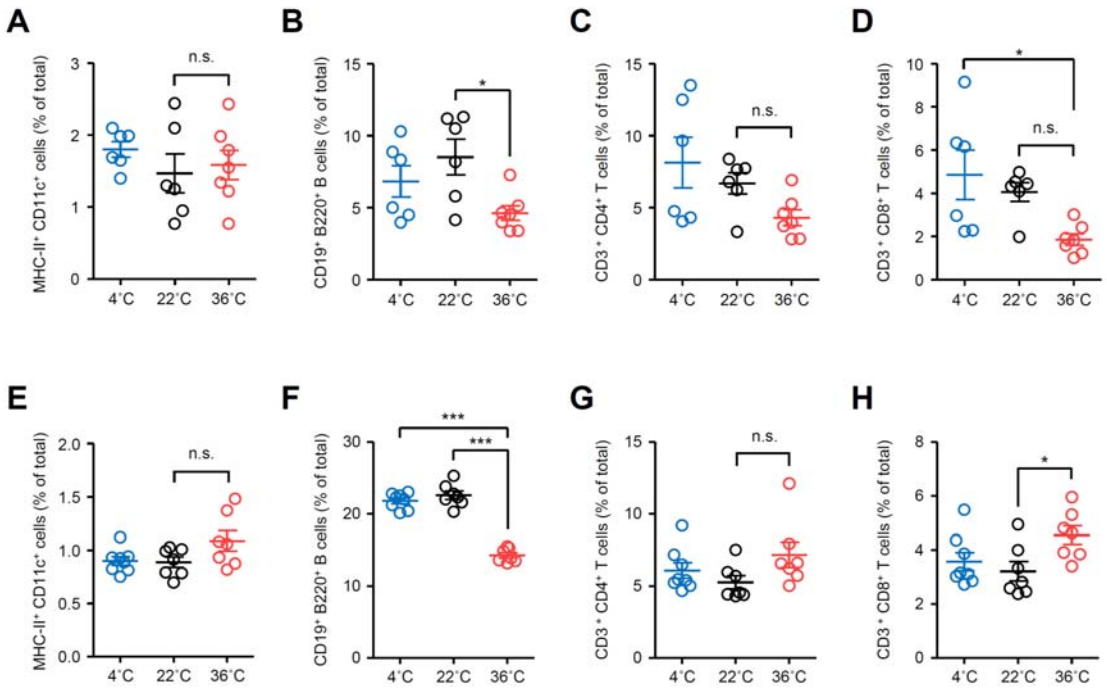

Figure S4. The effect of cold- or warm-exposure on cellularity in the lung of naïve mice. Mice were kept at 4, 22, or 36°C for 7 d. Frequency of MHC-II$^+$ CD11c$^+$ DCs, CD19$^+$ B220$^+$ B cells, CD3$^+$ CD4$^+$ T cells, and CD3$^+$ CD8$^+$ T cells in the lung (A-D) or spleen (E-H) are shown. *P < 0.05, ***P <0.001; n.s., not significant (one-way ANOVA and Tukey's test).

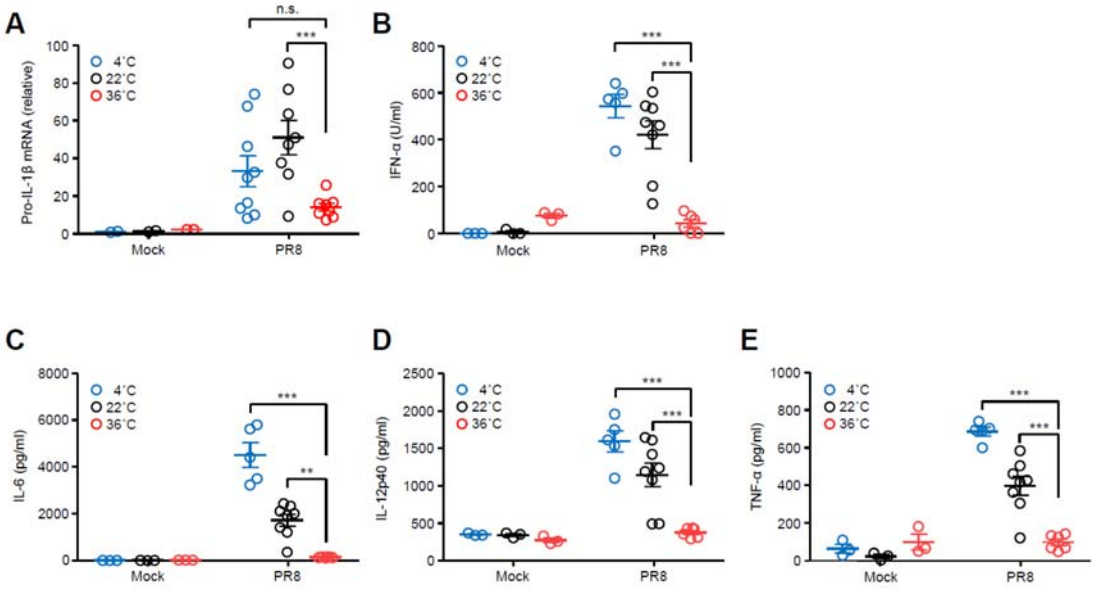

Figure S5. The effect of cold- or warm-exposure on cytokine production in the lung after intranasal influenza virus infection. Mice were kept at 4, 22, or 36˚C for 7 d before influenza virus infection. (A) Total RNAs were extracted from the lung of the mice at 0 and 48 h post infection. mRNA levels of pro-IL-1β were assessed by quantitative RT-PCR. GAPDH was used as an internal control. (B-E) The BALF was collected from influenza virus-infected animals at 2 d post infection. IFN-α (B), IL-6 (C), IL-12p40 (D), and TNF-α (E) levels in BALF were determined by ELISA. ***P* <0.01, ****P* <0.001; n.s., not significant (one-way ANOVA and Tukey's test).

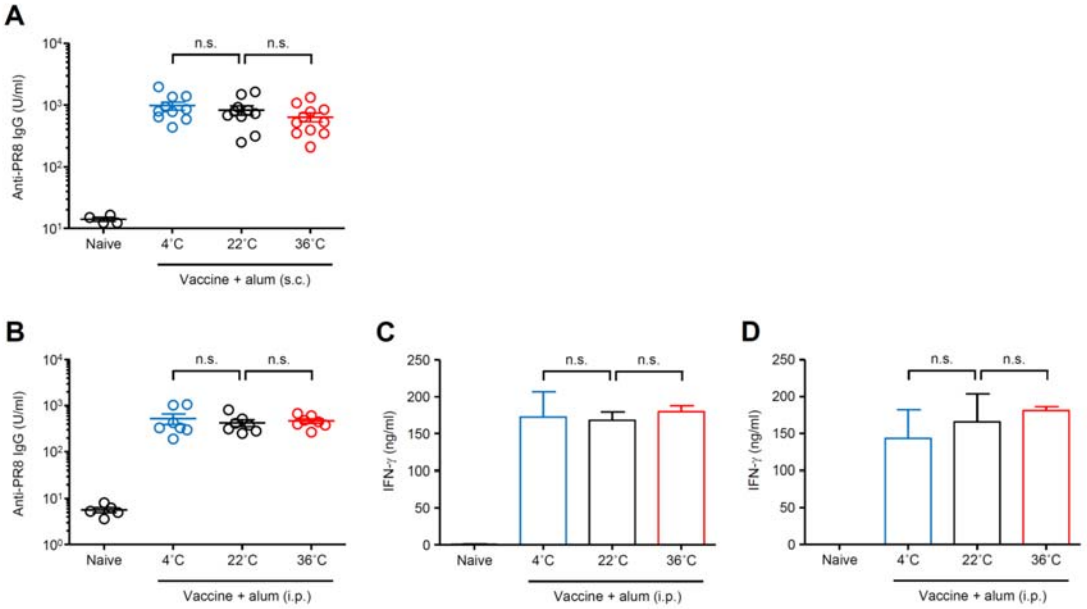

Figure S6. Warm exposure does not impair adaptive immune responses against immunization with influenza virus vaccine and aluminium adjuvant. Mice were kept at 4, 22, or 36˚C for 7 d before vaccination and throughout the experiment. (A) Mice were immunized s.c. with inactivated influenza virus vaccine and aluminum adjuvant. Ten days later, influenza virus-specific serum IgG levels were measured by ELISA. (B-D) Mice were immunized i.p. with inactivated influenza virus vaccine and aluminum adjuvant. (B) Two wks later, influenza virus-specific serum IgG levels were measured by ELISA. (C-D) Two wks later, $CD4^+$ T (C) or $CD8^+$ T (D) cells were isolated from spleen and stimulated with heat-inactivated influenza virus (C) or NP peptide (D) for 72 h, and IFN-γ production from $CD4^+$ T (C) or $CD8^+$ T (D) cells was measured by ELISA. n.s., not significant (one-way ANOVA and Tukey's test).

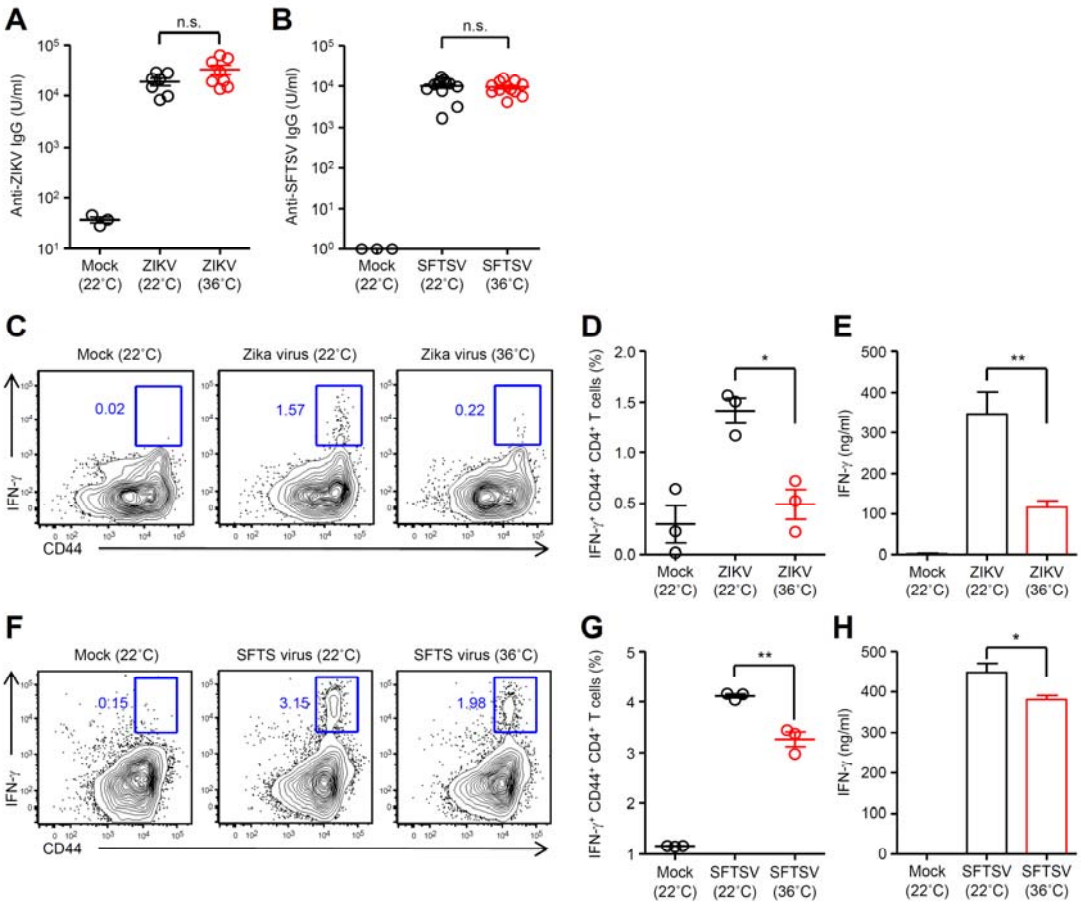

Figure S7. Impaired CD4$^+$ T cells response in high heat-exposed mice infected with ZIKV and SFTSV. Mice were kept at 22 or 36 °C for 7 d before i.p. infection with ZIKV (6×10^5 pfu per mouse) or SFTSV (6×10^6 $TCID_{50}$ per mouse) and throughout infection. (A-B) Serum were collected at 10 (A) or 14 (B) d post infection. ZIKV (A) or SFTSV (B)-specific serum IgG levels were measured by ELISA. (C-E) CD4$^+$ T cells were isolated from spleen and re-stimulated with heat-inactivated ZIKV for 72 h, and IFN-γ production from CD4$^+$ T cells was measured by intracellular IFN-γ staining (C and D) or by ELISA (E). Numbers adjacent to outlined areas indicate percent IFN-γ$^+$ CD44$^+$ cells among CD4$^+$ T cells (C). (F-H) CD4$^+$ T cells were isolated from spleen and re-stimulated with heat-inactivated SFTSV for 72 h, and IFN-γ production from CD4$^+$ T cells was measured by intracellular IFN-γ staining (F and G) or by ELISA (H). Numbers adjacent to outlined areas indicate percent IFN-γ$^+$ CD44$^+$ cells among CD4$^+$ T cells (F). The data are representative of two independent experiments (C and F) or are from two independent experiments (A, B, D, E, G and H; mean ± s.e.m.). *$P < 0.05$ and **$P < 0.01$; n.s., not significant (one-way ANOVA and Tukey's test).

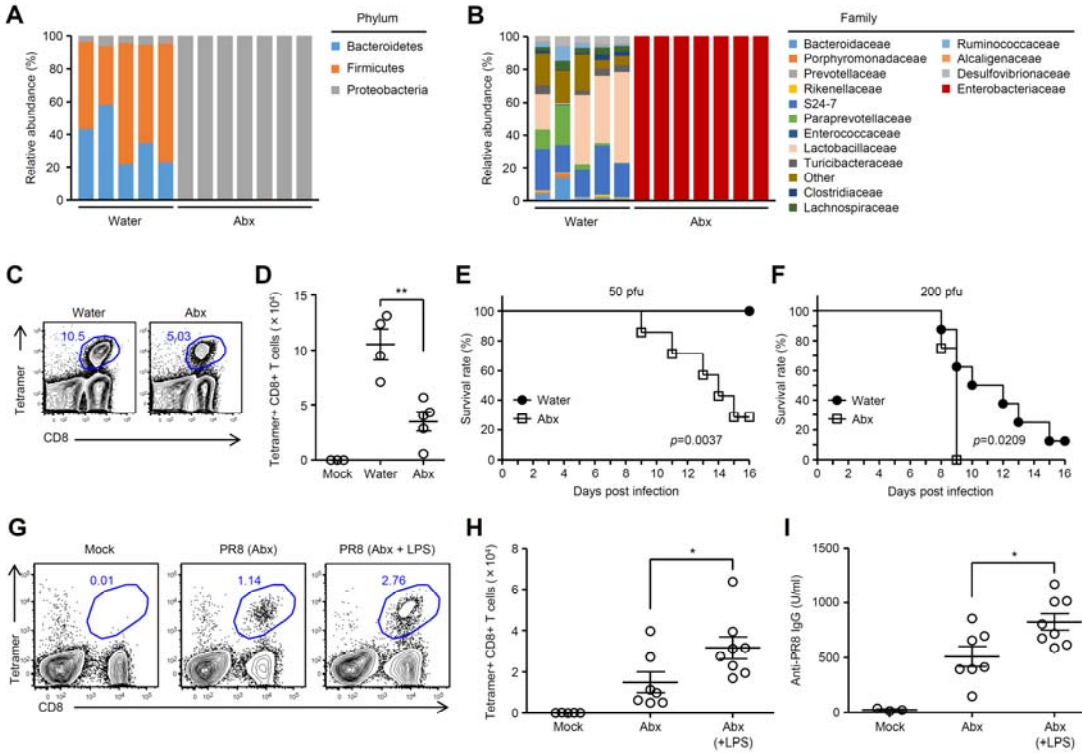

Figure S8. Impaired immune response to influenza virus infection in Abx-treated mice. (A-B) Comparison of phylum- (A) or family- (B) level proportional abundance of cecum of Abx-treated or control mice. (C-D) Mice were given antibiotics in drinking water for 4 wks before influenza virus infection (30 pfu per mouse) and throughout infection. Ten days later, lymphocytes were isolated from the lung. Influenza virus-specific $CD8^+$ T cells were then detected using the H-$2D^b$ influenza virus NP tetramer. (C) Numbers adjacent to outlined areas indicate percent tetramer-positive $CD8^+$ T cells. (D) Total numbers of influenza virus-specific $CD8^+$ T cells in the lung are shown. (E-F) Water-fed and Abx-treated mice were infected i.n. with 50 (E) or 200 (F) pfu of influenza virus. Survival of mice after infection is depicted. (G-I) Water-fed and Abx-treated mice were infected i.n. with 30 pfu of influenza virus with intrarectal injection of LPS (5 μg). Ten days later, lymphocytes were isolated from the lung. Influenza virus-specific $CD8^+$ T cells were then detected using the H-$2D^b$ influenza virus NP tetramer. (G) Numbers adjacent to outlined areas indicate percent tetramer-positive $CD8^+$ T cells. Total numbers of influenza virus-specific $CD8^+$ T cells in the lung (H) and influenza virus-specific serum IgG levels (I) are shown. $*P < 0.05$, $**P < 0.01$. Statistical significance was measured by log-rank (Mantel-Cox) test (E and F) or one-way ANOVA and Tukey's test (D, H and I).

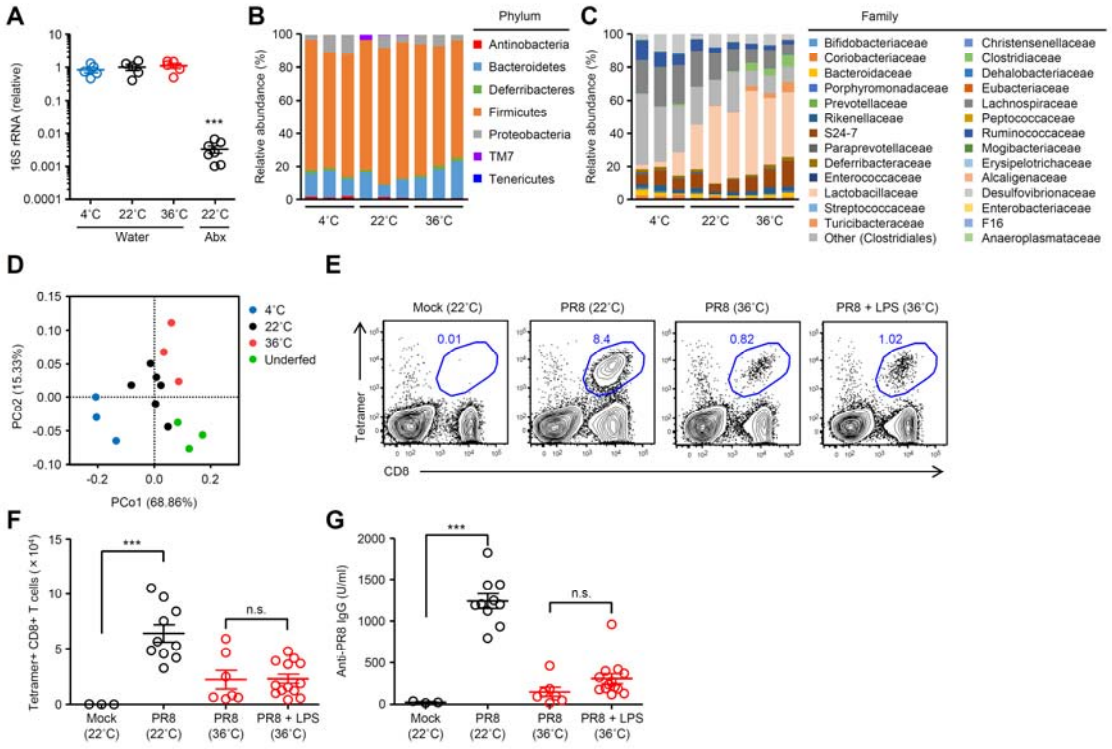

Figure S9. Warm exposure does not change the gut microbiota composition. (A) Mice were kept at 4, 22, or 36˚C for 7 d or were given antibiotics in drinking water for 4 wks. Relative gene copies of 16S rDNA isolated from stool pellets were quantified by qPCR. (B-C) Comparison of phylum- (B) or family- (C) level proportional abundance of cecum of cold-, RT-, and warm-exposed mice. (D) Principal coordinates analysis (PCoA) based on Weighted UniFrac analysis on operational taxonomic units (OTUs). Each symbol represents a single sample of cecum content of 7 days cold-exposed, high heat-exposed, or underfed mice (n = 3) and their RT-exposed (ad-libitum fed) controls (n = 6). (E-G) RT- and warm-exposed mice were infected i.n. with 30 pfu of influenza virus with or without intrarectal injection of LPS (5 μg). Ten days later, lymphocytes were isolated from the lung. Influenza virus-specific $CD8^+$ T cells were then detected using the $H\text{-}2D^b$ influenza virus NP tetramer. (E) Numbers adjacent to outlined areas indicate percent tetramer-positive $CD8^+$ T cells. Total numbers of influenza virus-specific $CD8^+$ T cells in the lung (F) and influenza virus-specific serum IgG levels (G) are shown. ***P <0.001; n.s., not significant (one-way ANOVA and Tukey's test).

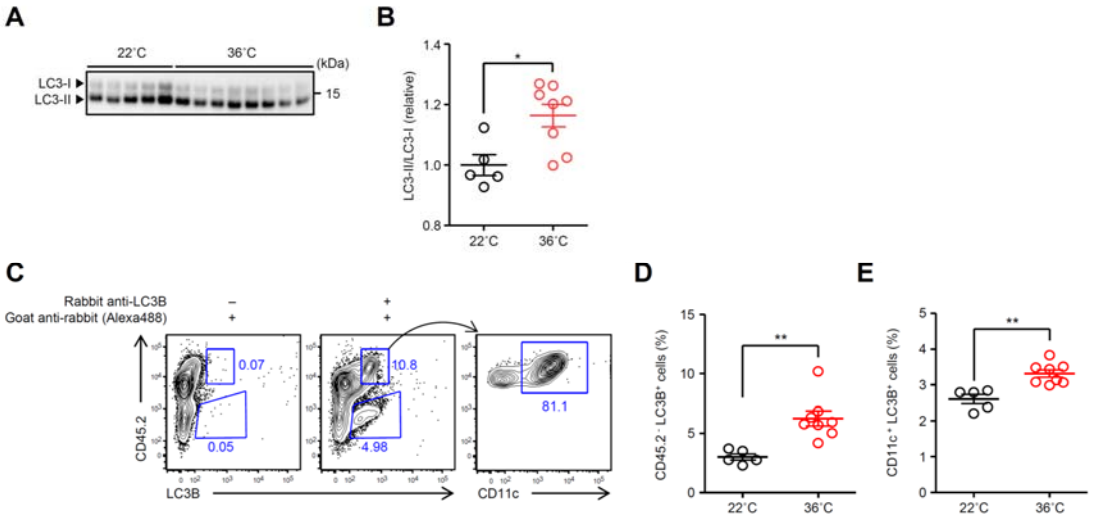

Figure S10. Induction of autophagy in the lung of high heat-exposed mice. Mice were kept at 22, or 36°C for 7 d. (A and B) BALF cell lysates were subjected to SDS-PAGE and analyzed by immunoblotting with rabbit polyclonal antibody against LC3B (#2775; Cell Signaling Technology). Relative expression levels of LC3-II normalized to LC3-I are shown (B). (C) The single-cell suspensions of lung samples from underfed mice were intracellularly stained with or without rabbit polyclonal antibody against LC3B (#2775; Cell Signaling Technology) followed by Alexa Fluor 488 goat anti-rabbit IgG (Invitrogen). (D and E) Percentages of CD45.2 negative $LC3B^+$ cells (D) or $CD45.2^+$ $CD11c^+$ $LC3B^+$ cells (E) in the lung are shown. Statistical analysis was performed by two-tailed Student's t test $*P < 0.05$, $**P < 0.01$.

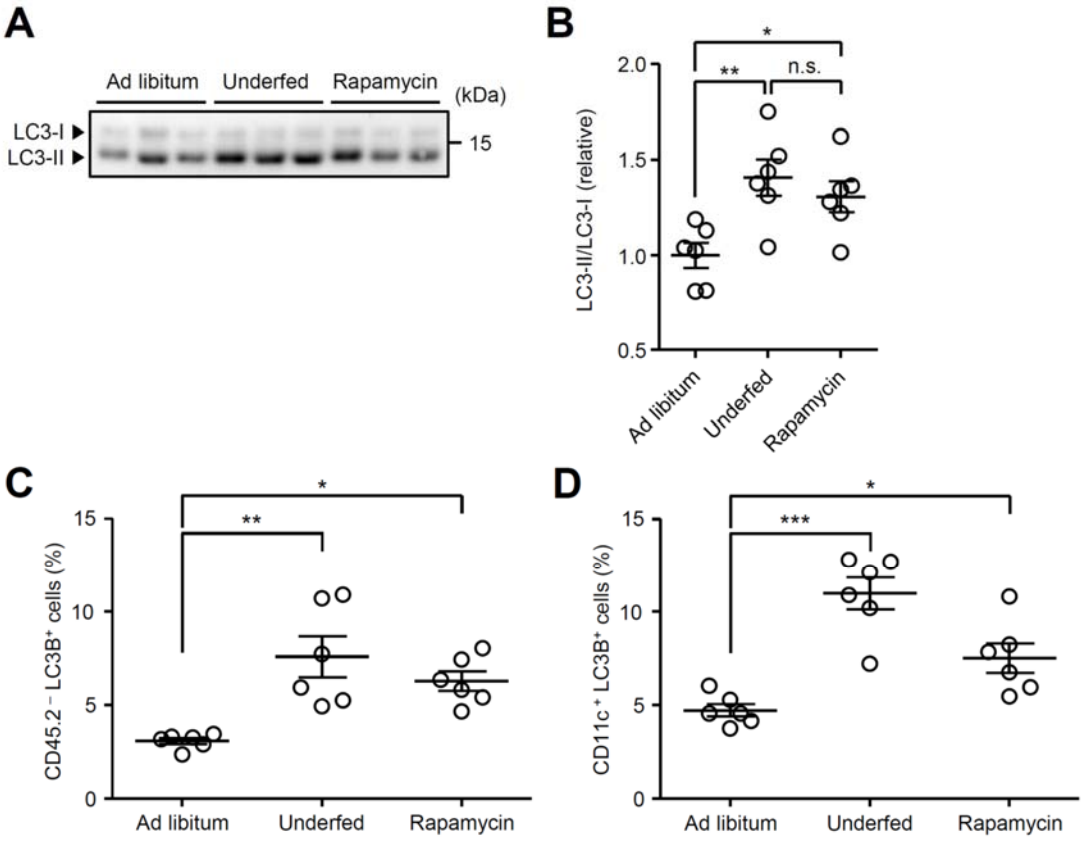

Figure S11. Induction of autophagy in the lung of underfed or rapamycin-treated mice. Mice were kept on food restricted or ad libitum-fed condition for 7 d. Rapamycin-treated group were administered i.v. with rapamycin daily for 2 d. (A and B) BALF cell lysates were subjected to SDS-PAGE and analyzed by immunoblotting with rabbit polyclonal antibody against LC3B (#2775; Cell Signaling Technology). Relative expression levels of LC3-II normalized to LC3-I are shown (B). (C and D) The single-cell suspensions of lung samples were intracellularly stained with rabbit polyclonal antibody against LC3B (#2775; Cell Signaling Technology) followed by Alexa Fluor 488 goat anti-rabbit IgG (Invitrogen). Percentages of CD45.2 negative $LC3B^+$ cells (C) or $CD45.2^+$ $CD11c^+$ $LC3B^+$ cells (D) in the lung are shown. **P* <0.05, ***P* <0.01, ****P* <0.001; n.s., not significant (one-way ANOVA and Tukey's test).

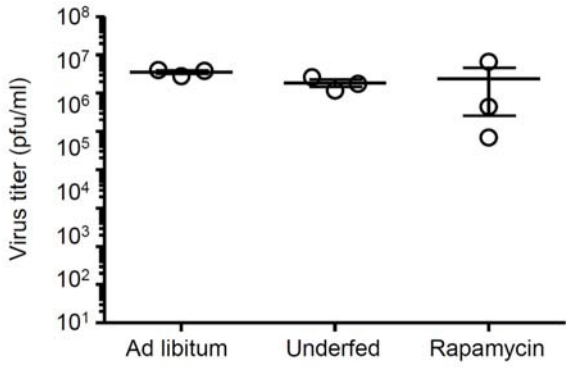

Figure S12. Influenza virus replication in the lung of ad libitum-fed, underfed, or rapamycin-treated mice. Mice were kept on food restricted or ad libitum-fed condition for 7 d before influenza virus infection (1,000 pfu per mouse) and throughout infection. Rapamycin-treated group were administered i.v. with rapamycin daily from day -2 to day 4 during infection. The BALF were collected at 5 d post infection and virus titers were measured by plaque assay.

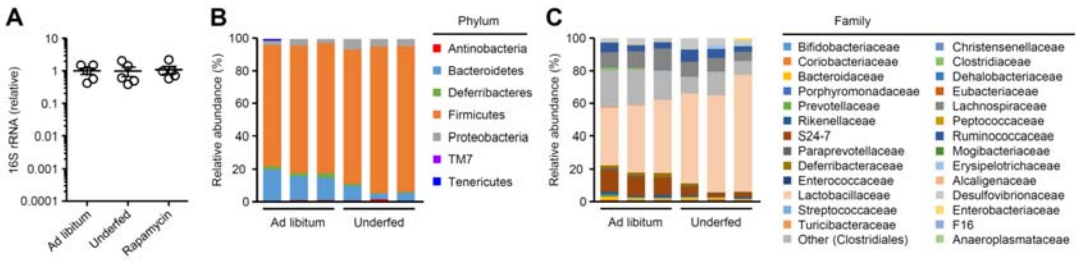

Figure S13. Starvation does not change the gut microbiota composition. (A) Mice were kept on food restricted or ad libitum-fed condition for 7 d. Rapamycin-treated group were administered i.v. with rapamycin daily for 2 d. Relative gene copies of 16S rDNA isolated from stool pellets were quantified by qPCR. (B-C) Comparison of phylum- (B) or family- (C) level proportional abundance of cecum of ad libitum-fed, underfed, and rapamycin-treated mice.

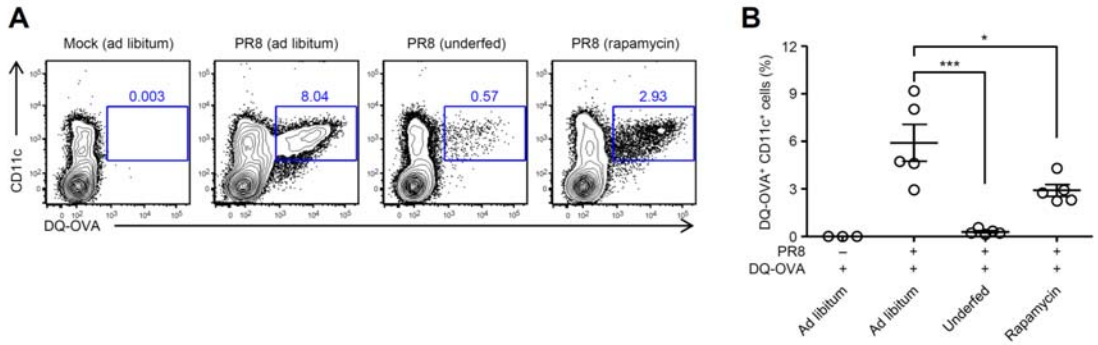

Figure S14. Respiratory tract DCs fail to migrate to the draining LN in underfed or rapamycin-treated mice. Mice were kept on food restricted or ad libitum-fed condition for 7 d before influenza virus infection (1,000 pfu per mouse). Rapamycin-treated group were administered i.v. with rapamycin daily for 2 d before influenza virus infection (1,000 pfu per mouse). These mice were inoculated intranasally with DQ-OVA. Six hours later, mice were infected with 1,000 pfu of PR8 viruses. Eighteen hours after infection, mediastinal LNs were collected. Numbers adjacent to outlined areas indicate percent DQ-OVA$^+$ CD11c$^+$ DCs cells (A). Percentages of DQ-OVA$^+$ CD11c$^+$ DCs are shown (B). *P <0.05, ***P <0.001 (one-way ANOVA and Tukey's test).

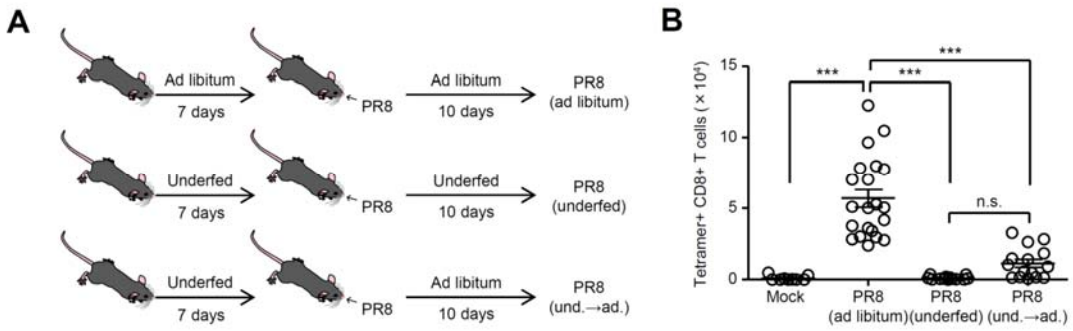

Figure S15. Ad libitum self-feeding condition after infection with influenza virus is insufficient to restore immune response in underfed mice. (A) Mice were kept on food restricted or ad libitum-fed condition for 7 d before influenza virus infection (30 pfu per mouse) and throughout infection. Some underfed mice were kept on ad libitum-fed condition after infection. (B) Ten days later, lymphocytes were isolated from the lung. Influenza virus-specific $CD8^+$ T cells were then detected using the H-$2D^b$ influenza virus NP tetramer. Total numbers of influenza virus-specific $CD8^+$ T cells in the lung are shown. The data are pooled from three independent experiments. ***P <0.001 (one-way ANOVA and Tukey's test).

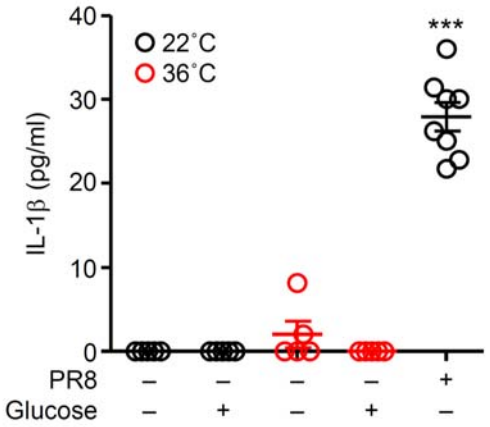

Figure S16. Intravenous injection with glucose alone failed to stimulate IL-1β secretion in the lung. Mice were kept 22 or 36°C for 7 d. IL-1β levels in BALF of RT- or high heat-exposed mice 2 days after intravenous inoculation with glucose on day 0 and day 1. Influenza virus strain A/PR8 serves as a positive control. IL-1β levels in BALF were determined by ELISA. ****P* <0.001 (one-way ANOVA and Tukey's test).

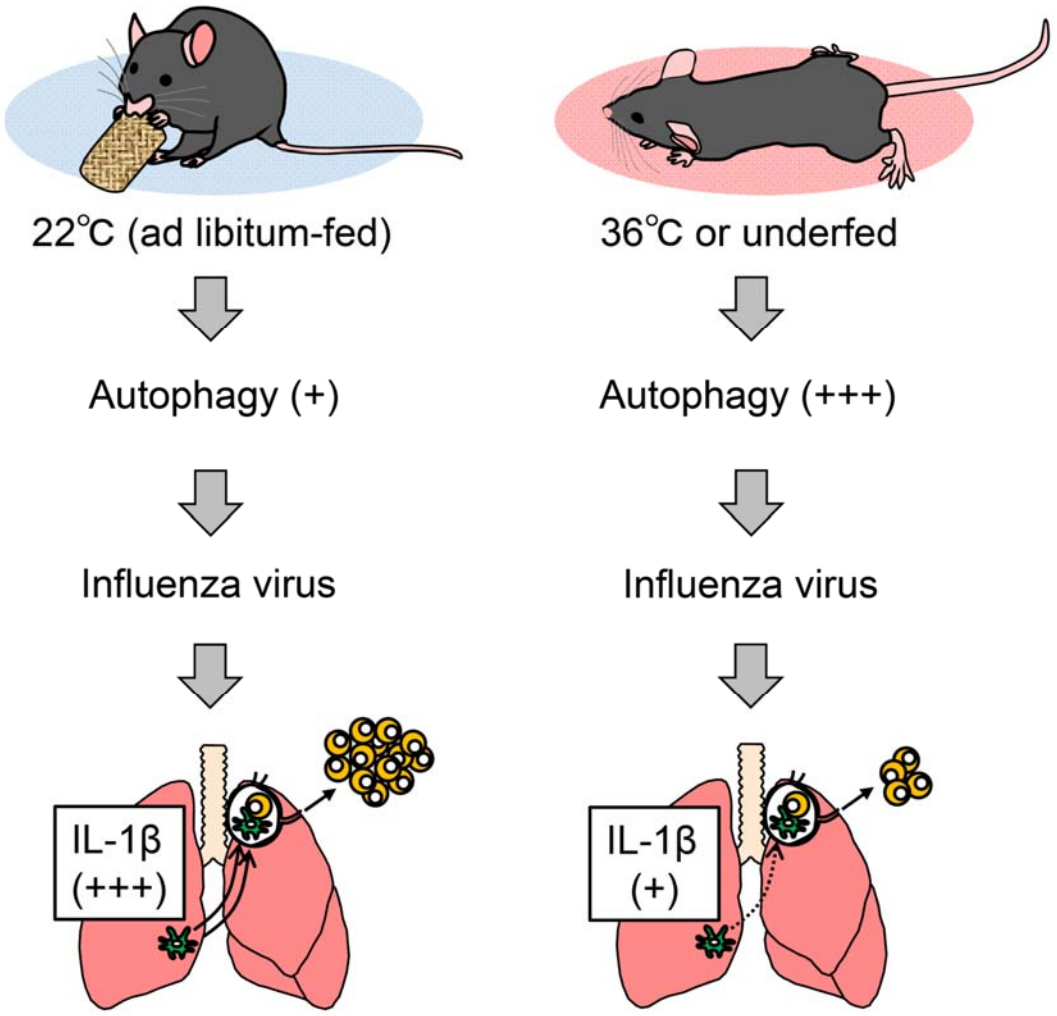

Figure S17. Proposed mechanism by which high ambient temperature dampen adaptive immune responses to influenza virus infection. Reduced feeding behavior increases autophagy in the lung of high heat-exposed or underfed mice at steady state. As a result of increased autophagy in the lung, inflammasome-dependent IL-1β secretion in the lung and migration of antigen-captured lung DCs to the mLN are severely impaired in high heat-exposed or underfed mice following influenza virus infection. Administration of glucose or dietary SCFAs such as butyrate, propionate, and acetate could restore influenza virus-specific adaptive immune responses in high heat-exposed mice.

Supplementary References

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