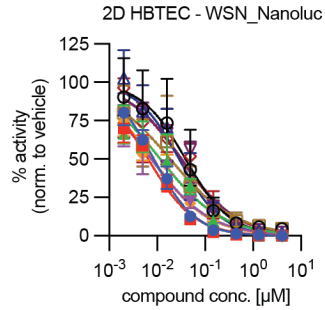


1 Supporting information captions



- HBTEC "F1" - EC₅₀ - 0.009 μM; EC₉₀ - 0.084 μM
- HBTEC "F2" - EC₅₀ - 0.007 μM; EC₉₀ - 0.093 μM
- ▲ HBTEC "F3" - EC₅₀ - 0.012 μM; EC₉₀ - 0.749 μM
- ▼ HBTEC "F4" - EC₅₀ - 0.008 μM; EC₉₀ - 0.285 μM
- ◆ HBTEC "F5" - EC₅₀ - 0.008 μM; EC₉₀ - 0.241 μM
- HBTEC "M2" - EC₅₀ - 0.039 μM; EC₉₀ - 0.361 μM
- HBTEC "M3" - EC₅₀ - 0.019 μM; EC₉₀ - 0.646 μM
- △ HBTEC "M4" - EC₅₀ - 0.029 μM; EC₉₀ - 0.249 μM
- ▽ HBTEC "M5" - EC₅₀ - 0.032 μM; EC₉₀ - 0.988 μM
- ◇ HBTEC "M6" - EC₅₀ - 0.031 μM; EC₉₀ - 1.024 μM

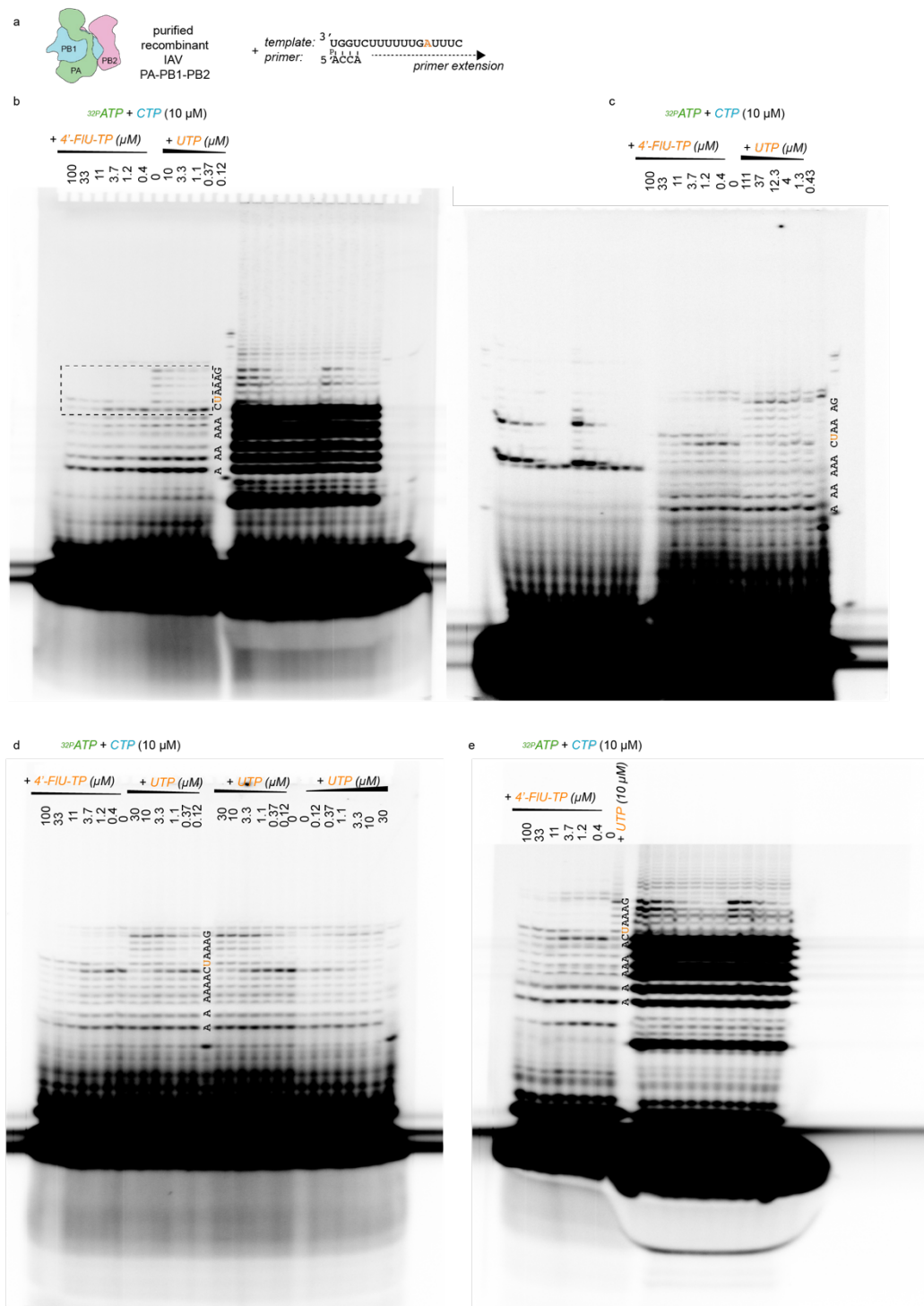
2

3 **S1 Figure:** 4'-FIU antiviral activity in 2D HBTEC. Dose-response assays of 4'-FIU against A/WSN/33

4 (H1N1) on HBTEC derived from 10 different healthy donors, five male and five female. Lines represent

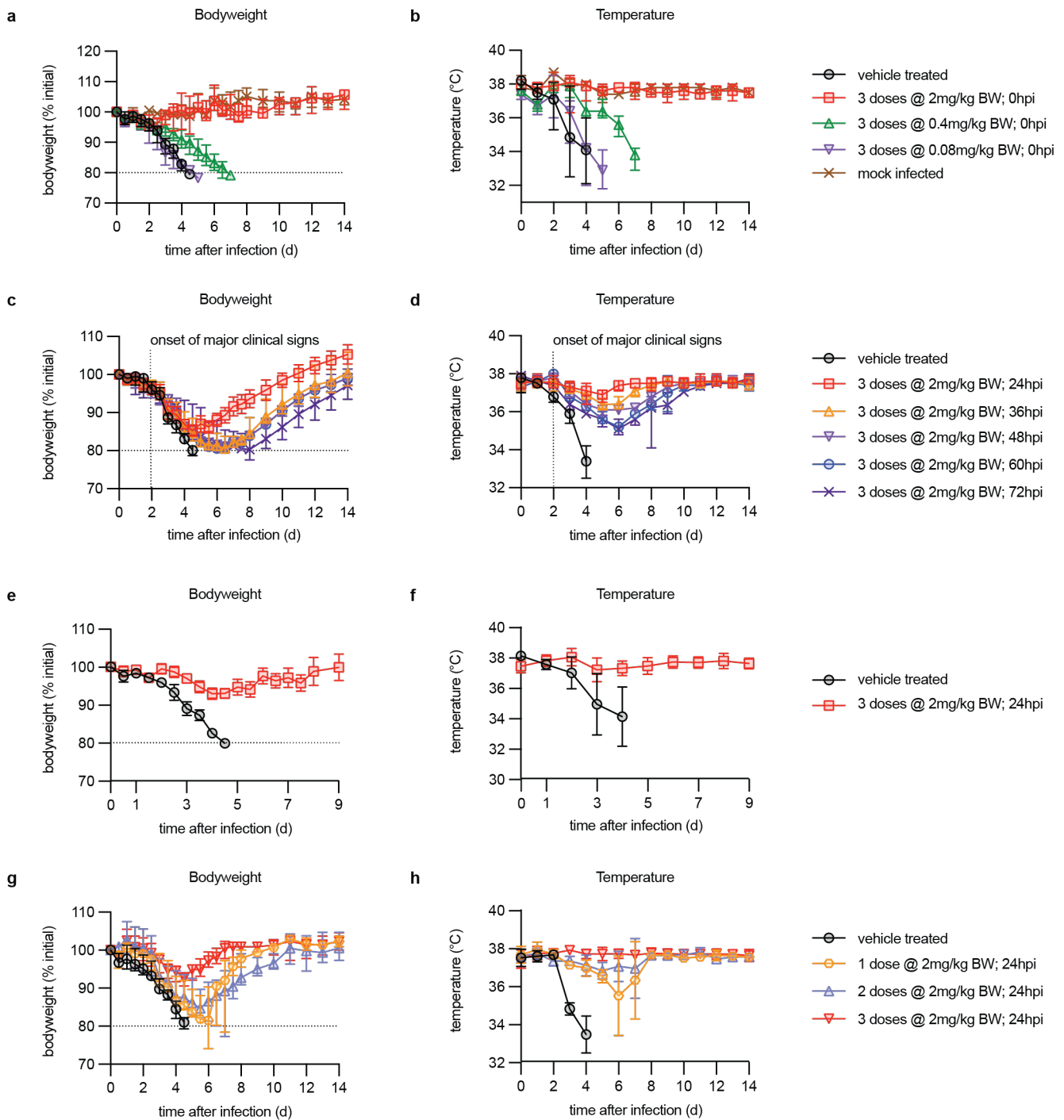
5 4-parameter variable slope regression models; symbols represent data means \pm SD; n=6. EC₅₀

6 concentrations and 95% CI are specified.



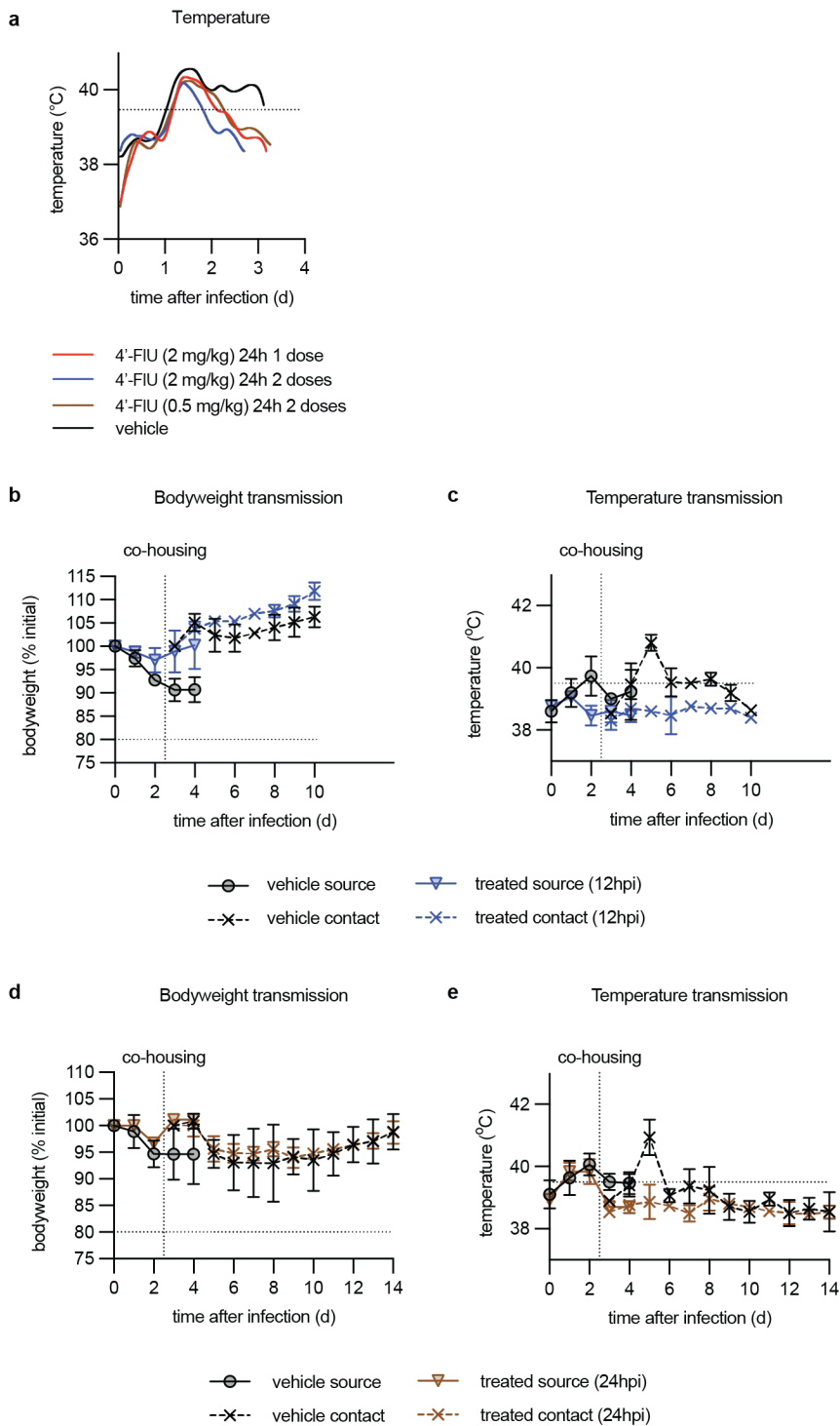
7

8 **S2 Figure:** Urea-PAGE fractionation (uncropped) of RNA transcripts. **a)** Template and primer used in
 9 the reaction. **b-e)** Independent repeats of primer extension assay by IAV polymerase. Dashed rectangle
 10 shows insert presented in figure 1e-f. Replicates were included in quantitations presented in figure 1e-f.



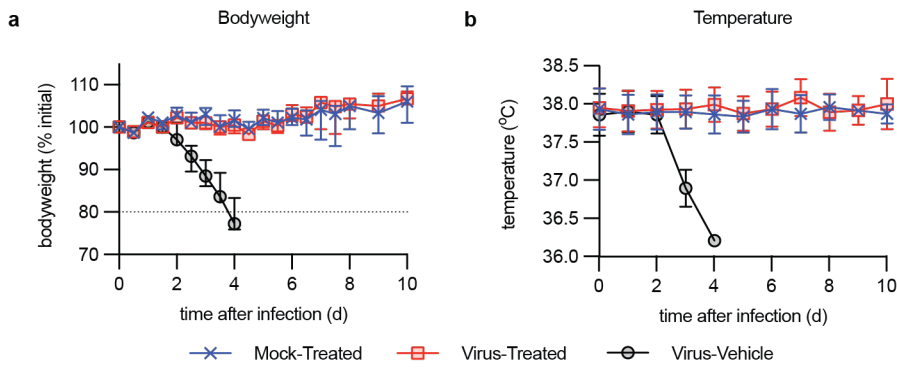
11

12 **S3 Figure:** Clinical signs in 4¹-FIU-treated mice used to establish treatment paradigms. **a-h)** Body
 13 weight measurements taken twice daily (a, c, e, g) and rectal body temperature determined once daily
 14 (b, d, f, h). Dashed horizontal line specifies humane endpoint of 20% body weight loss; symbols
 15 represent data means \pm SD; n values are specified in main figure 2. Results are shown for lowest
 16 efficacious dose (a-b), latest onset of efficacious treatment (c-d), virus clearance (e-f) and minimal
 17 number of doses required (g-h) studies.

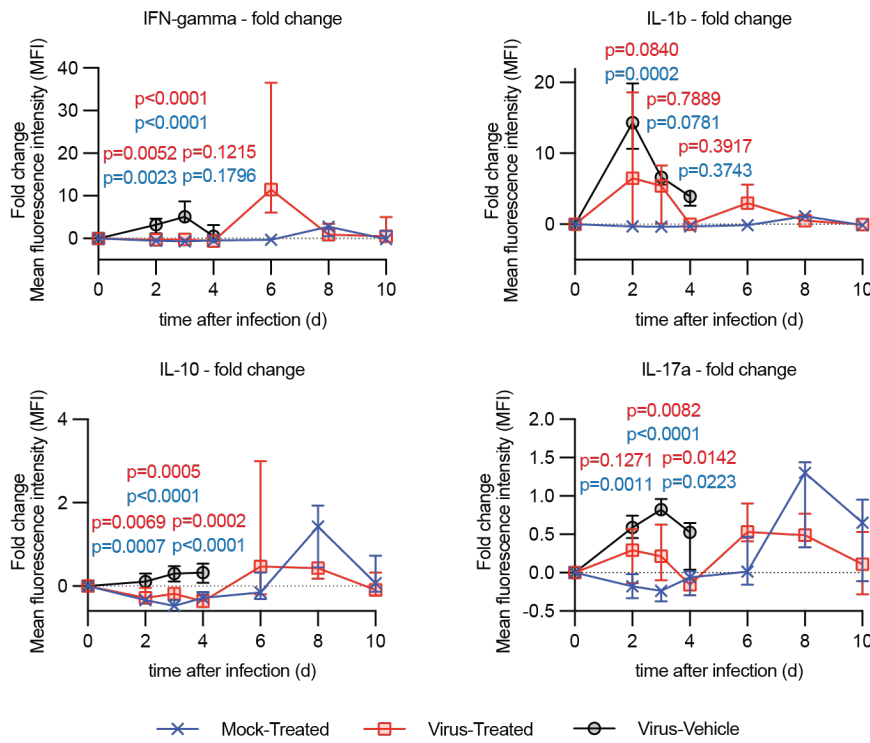


18

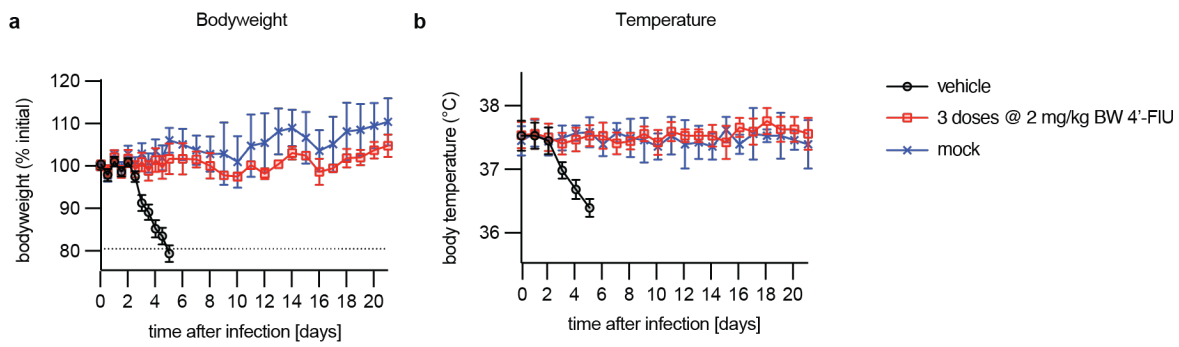
19 **S4 Figure:** Clinical signs in 4'-FIU treated ferrets. **a)** Continuous telemetric assessment of body
 20 temperature of ferrets in the 4'-FIU efficacy study. **b-e)** Once-daily body weight (b, d) and rectal body
 21 temperature (c, e) of ferrets involved in the Ca09 transmission studies.



22
 23 **S5 Figure:** Clinical signs in 4'-FIU-treated mice used in immune response-mitigation study. **a-b)** Body
 24 weight measurements taken twice daily (a), and rectal body temperature determined once daily (b).
 25 Symbols represent data means \pm SD; n values are specified in figure 4.



26
 27 **S6 Figure:** Changes in select BALF cytokine levels in infected and 4'-FIU-treated mice. IFN γ , IL-1b, IL-
 28 10, and IL-17a levels present in BALF of animals treated as in figure 4a are shown, calculated relative
 29 to levels at time of infection. Symbols represent data medians with 95% CI; 2-way ANOVA with Tukey's
 30 *post hoc* test; P values are specified.

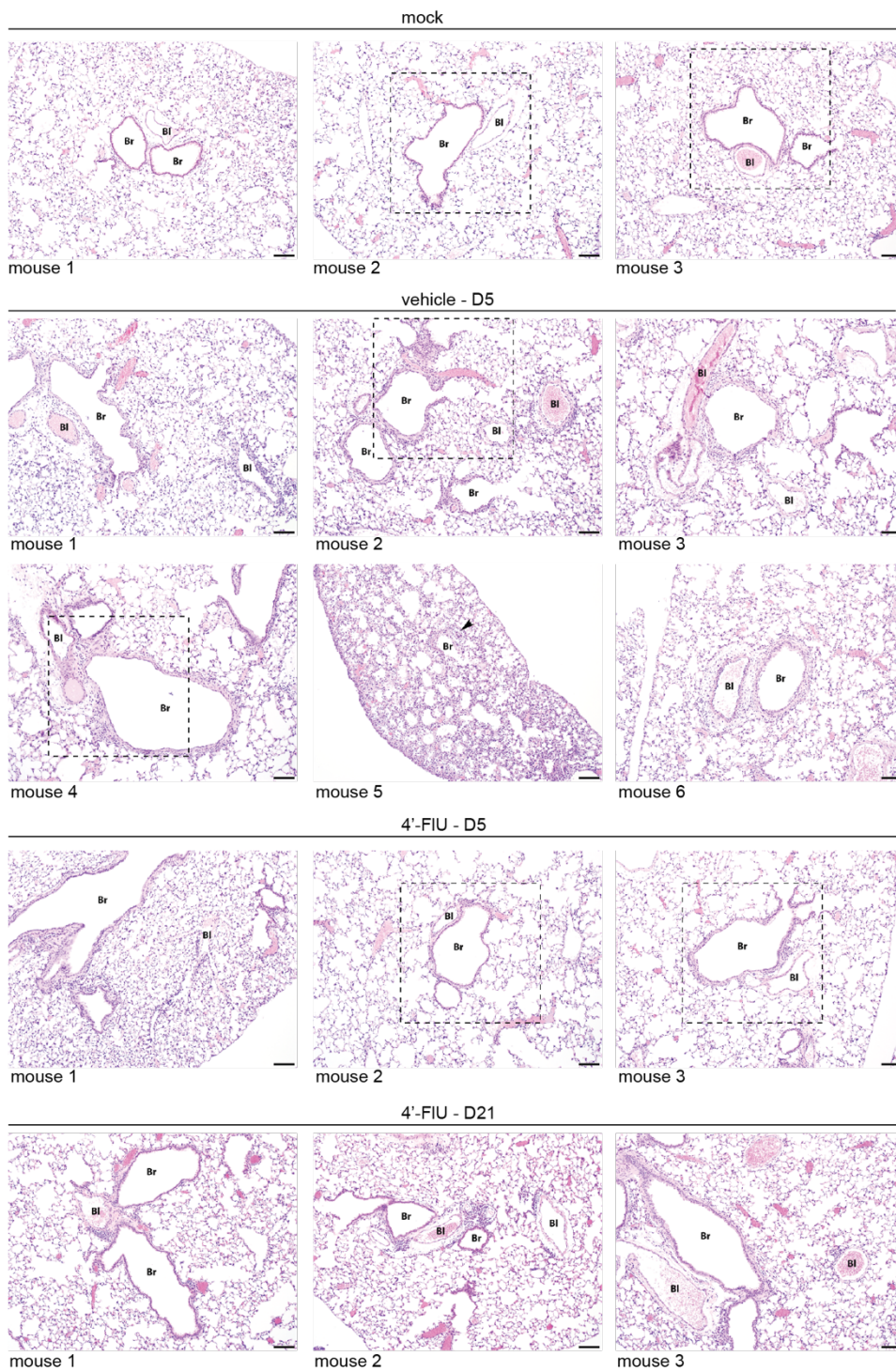


31

32 **S7 Figure:** Clinical signs of mice involved in mitigation of histopathology study. **a-b)** Body weight

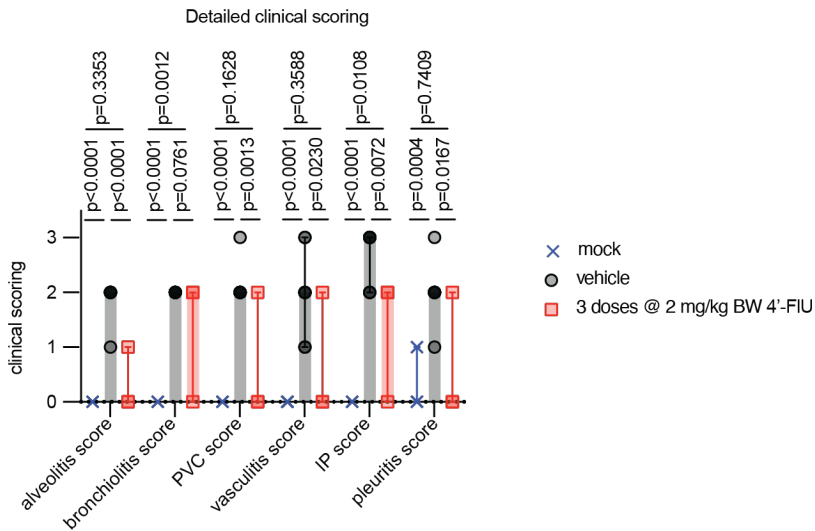
33 measurements taken twice daily (a), and rectal body temperature determined once daily (b). Symbols

34 represent data means \pm SD.



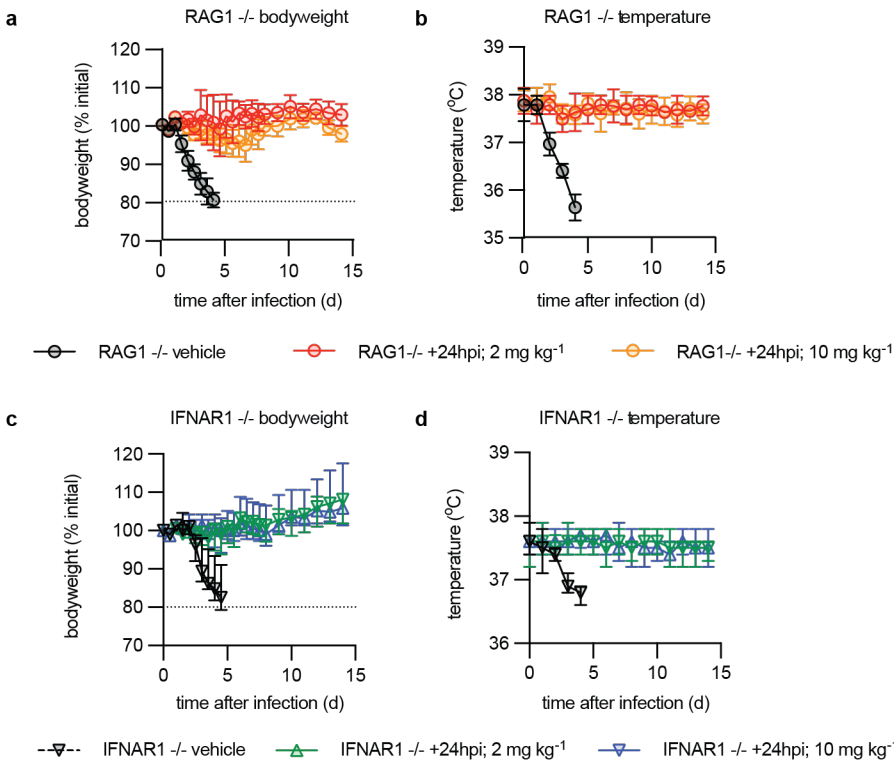
35

36 **S8 Figure:** Photomicrographs of lung tissues. Lungs were extracted on study days 5 or 21 (infected
 37 and treated animals only) of all animals examined in this study; tissue section were H&E stained;
 38 dashed rectangle shows sections presented in figure 4e.; magnification 10×; scale bar 100 μm; Br,
 39 bronchiole; Bl or arrowhead, blood vessel.



40

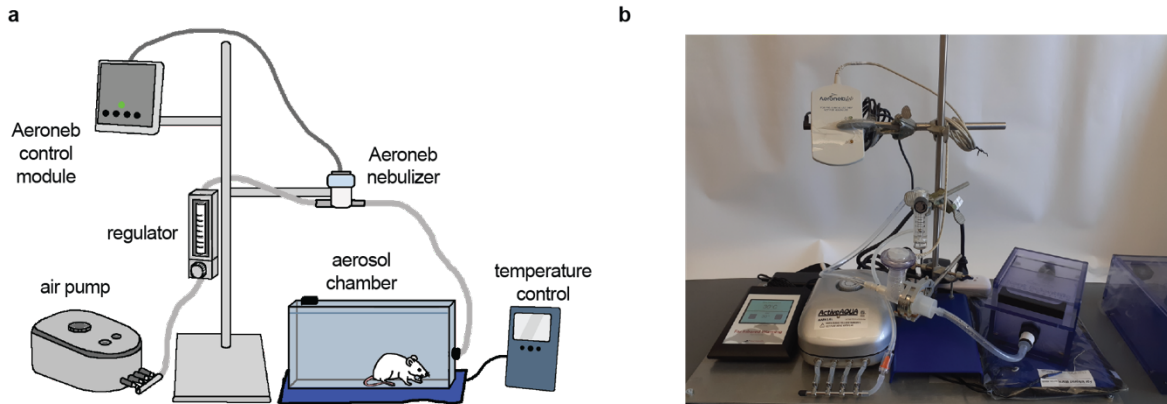
41 **S9 Figure:** Detailed histopathological scores of all animals examined in this study. Individual clinical
 42 scores for alveolitis, bronchiolitis, perivascular cuffing (PVC), vasculitis, interstitial pneumonia (IP), and
 43 pleuritis. Columns show data medians, symbols represent individual animals; 1-way ANOVA with
 44 Tukey's *post hoc* test; p values are specified; n values are specified in figure 4f.



45

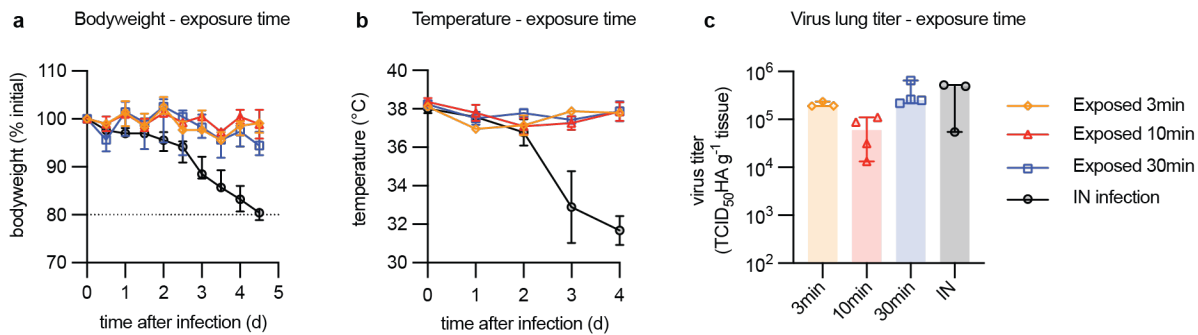
46 **S10 Figure:** Clinical signs in Ca09-infected immunocompromised mice treated with 4'-FIU. **a-c)** Shown
 47 are body weight measurements taken twice daily (a, c), and rectal body temperature (b, d) determined

48 once daily. Symbols represent data means \pm SD; n values are specified in figure 5. Results are shown
 49 for RAG1 KO (a-b) and IFNAR1 KO (c-d) animals.



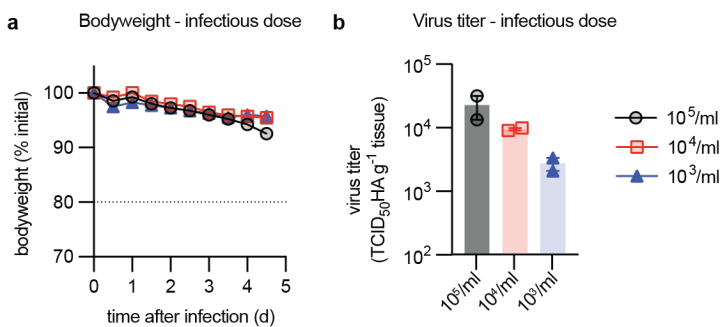
50

51 **S11 Figure:** Set-up of the Aeroneb nebulizer infection system. **a-b)** Schematic representation of the
 52 system (a) and photograph of the assembly (b).



53

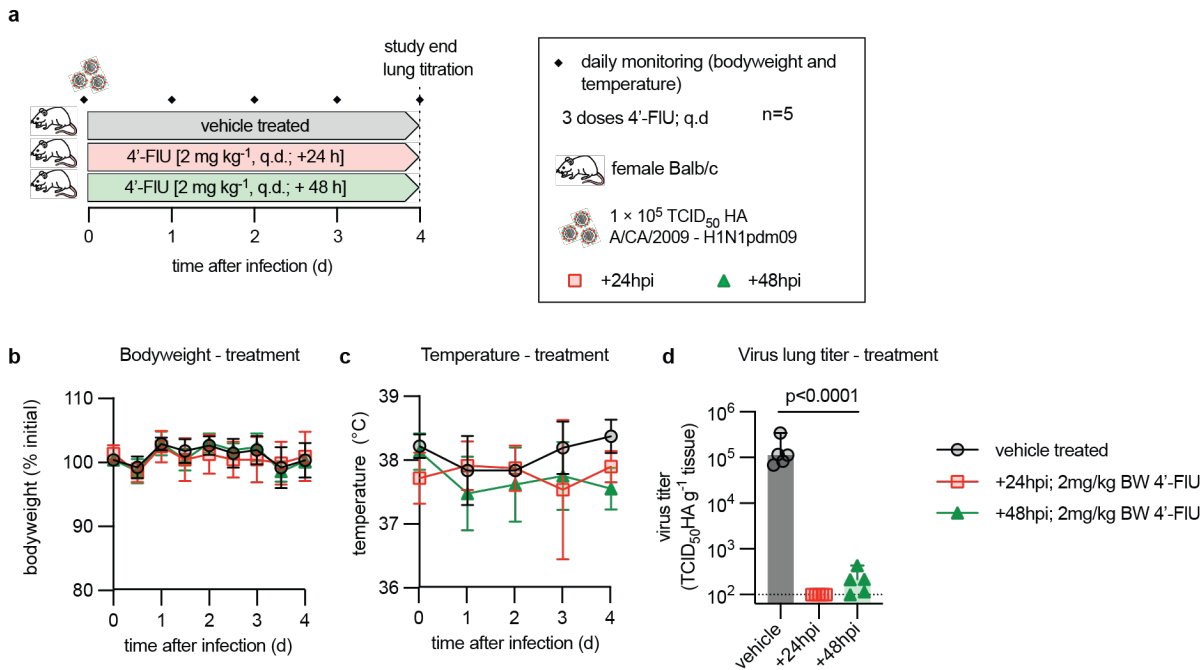
54 **S12 Figure:** S12. Variation of duration of animal exposure in the aerosolization chamber. **a-b)** Body
 55 weight measurements taken twice daily (a), and rectal body temperature (b) determined once daily.
 56 Symbols represent data means \pm SD. **c)** Lung viral titers determined 4 days after infection. Columns
 57 represent data medians with 95% CI; symbols show individual animals; n=4.



58

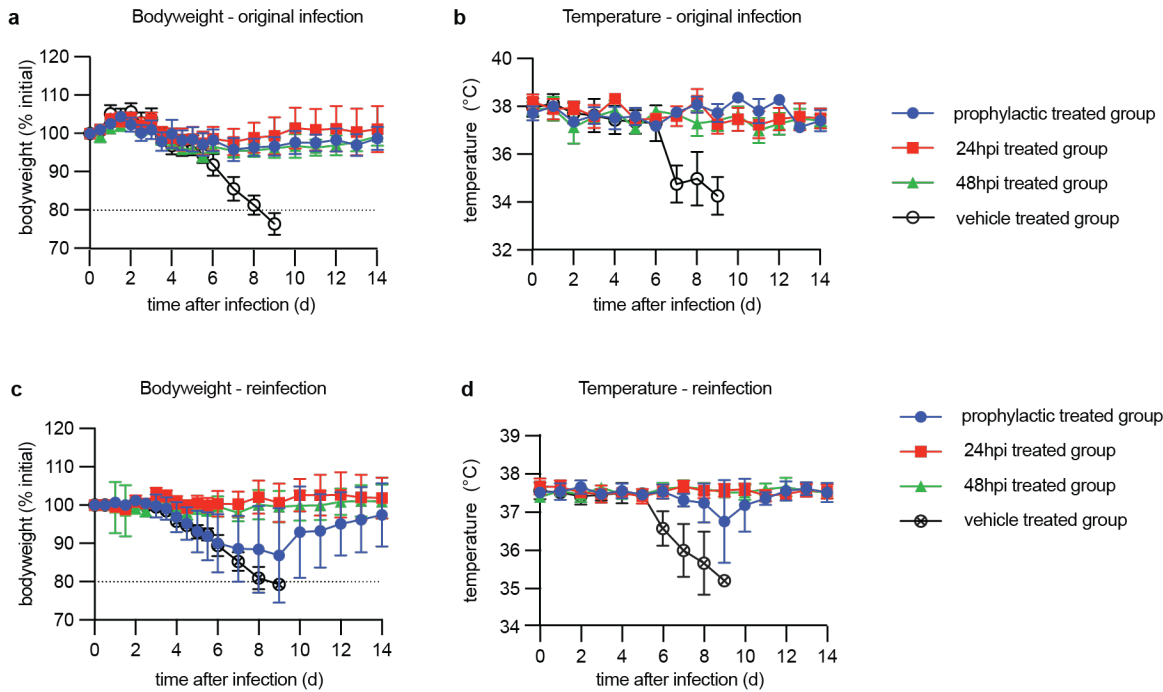
59 **S13 Figure:** Variation of Ca09 inoculum amount for infection in the aerosolization chamber. **a)** Body
 60 weight measurements taken twice daily. Symbols represent data means \pm SD. **b)** Lung viral titers

61 determined 4 days after infection. Columns represent data mean with range; symbols show individual
 62 animals; n=2.



63

64 **S14 Figure:** 4'-FIU efficacy confirmation after infection with aerosolized inoculum virus. **a)** Efficacy
 65 study schematic; n values are specified. **b-c)** Body weight measurements of animals from (a) taken
 66 twice daily (a), and rectal body temperature (b) determined once daily. Symbols represent data means
 67 ± SD. **d)** Lung viral titers determined 4 days after infection. Columns represent data median with 95%
 68 CI, symbols show individual animals; 1-way ANOVA with Dunnett's *post hoc* test; P values are
 69 specified.



70

71 **S15 Figure:** Clinical signs of mice infected and reinfected with Ca09. **a-d)** Body weight measurements
 72 taken twice daily (a, c), and rectal body temperature determined once daily (b, d). Symbols represent
 73 data means \pm SD. Results are shown for animals after the original infection (a-b) and after homotypic
 74 reinfection (c-d).

75 **S1 Data.** All quantitative source data generated in this study.

76 **S2 Data.** All statistical analyses performed for this study.