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SUPPLEMENTARY INFORMATION

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Ferret animal model of severe fever with thrombocytopenia syndrome phlebovirus for human lethal infection and pathogenesis

Su-Jin Park[1,2,5], Young-Il Kim[1,2,5], Angela Park[3], Hyeok-Il Kwon[1,2], Eun-Ha Kim[1,2], Young-Jae Si[1,2], Min-Suk Song[1,2], Chul-Ho Lee[4], Kyle Jung[3], Woo-Jin Shin[3], Jianxiong Zeng[3], Younho Choi[3], Jae U. Jung[3]* and Young Ki Choi[1,2]*

[1]Department of Microbiology, College of Medicine and Medical Research Institute, Chungbuk National University, Cheongju, Republic of Korea. [2]Zoonotic Infectious Diseases Research Center, Chungbuk National University, Cheongju, Republic of Korea. [3]Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA. [4]Laboratory Animal Center, Korea Research Institute of Bioscience and Biotechnology, University of Science and Technology, Daejeon, Republic of Korea. [5]These authors contributed equally: Su-Jin Park, Young-Il Kim. *e-mail: jaeujung@med.usc.edu; choiki55@chungbuk.ac.kr

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[1]Department of Microbiology, College of Medicine and Medical Research Institute, Chungbuk National University, Cheongju, Republic of Korea

[2]Zoonotic Infectious Diseases Research Center, Chungbuk National University, Cheongju, Korea

[3]Department of Molecular Microbiology and Immunology, Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA

[4]Laboratory Animal Center, Korea Research Institute of Bioscience and Biotechnology, University of Science and Technology, Daejeon, Republic of Korea

Short title: Fatal animal model of SFTSV infection.

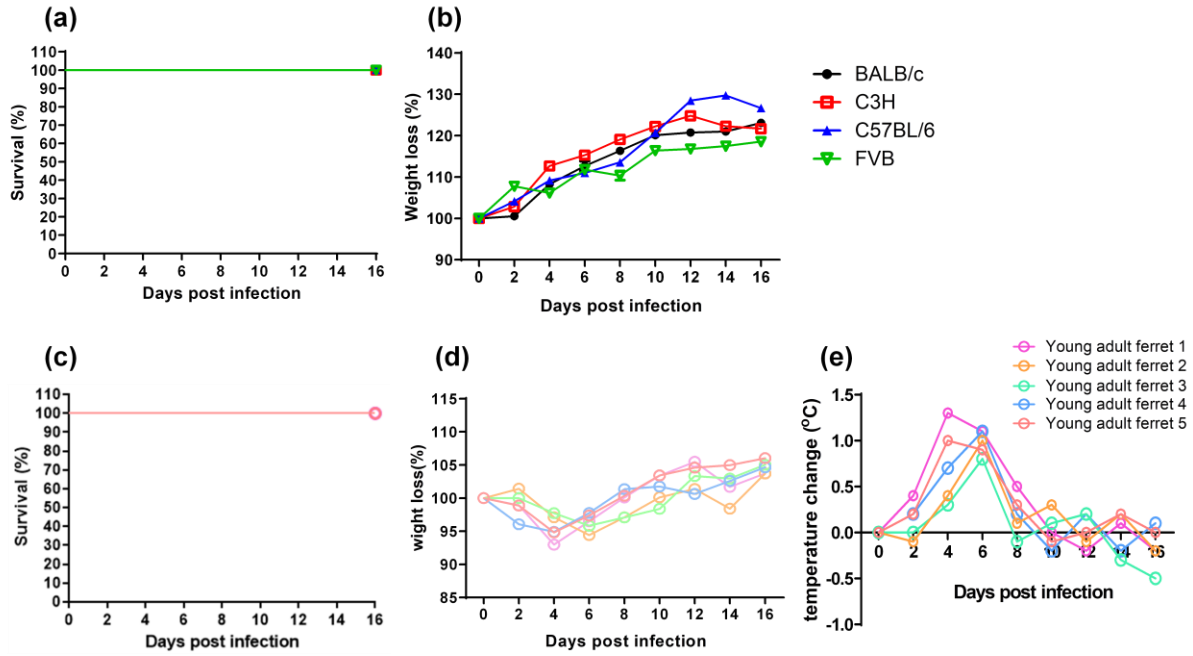
[5]These authors contributed equally to this work as co-first authors.

* Corresponding authors: Jae U. Jung*[3], and Young Ki Choi[1]*

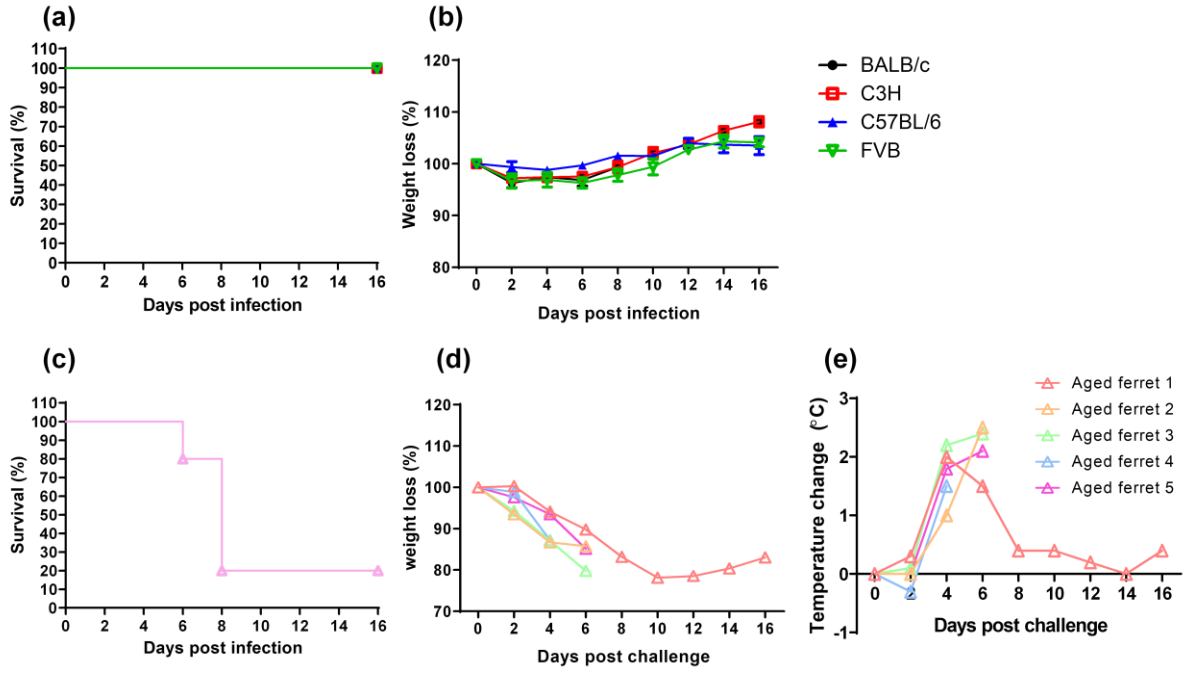
[3]Department of Molecular Microbiology and Immunology,
Keck School of Medicine, University of Southern California, Los Angeles, CA 90033, USA
E-mail: jaeujung@med.usc.edu

[1]Department of Microbiology, College of Medicine and Medical Research Institute
Chungbuk National University, 1 Chungdae-ro, Seowon-gu, Cheongju.28644, South Korea
Tel: 82-43-261-3384
E-mail: choiki55@chungbuk.ac.kr

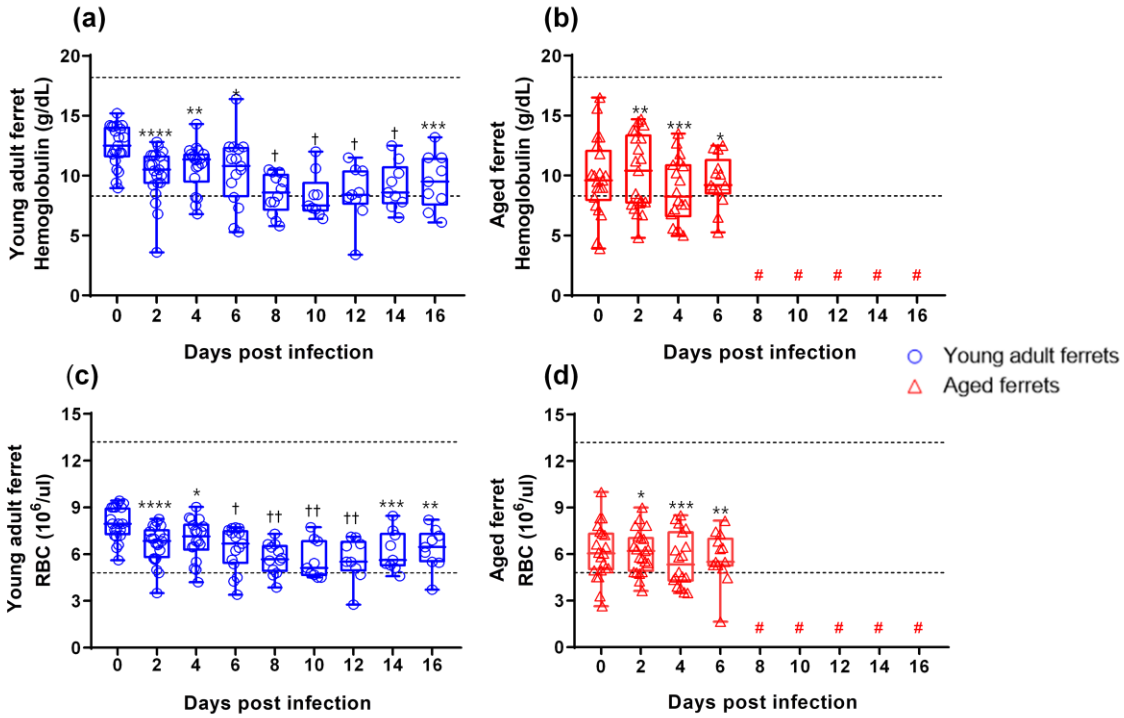
Supplemental information



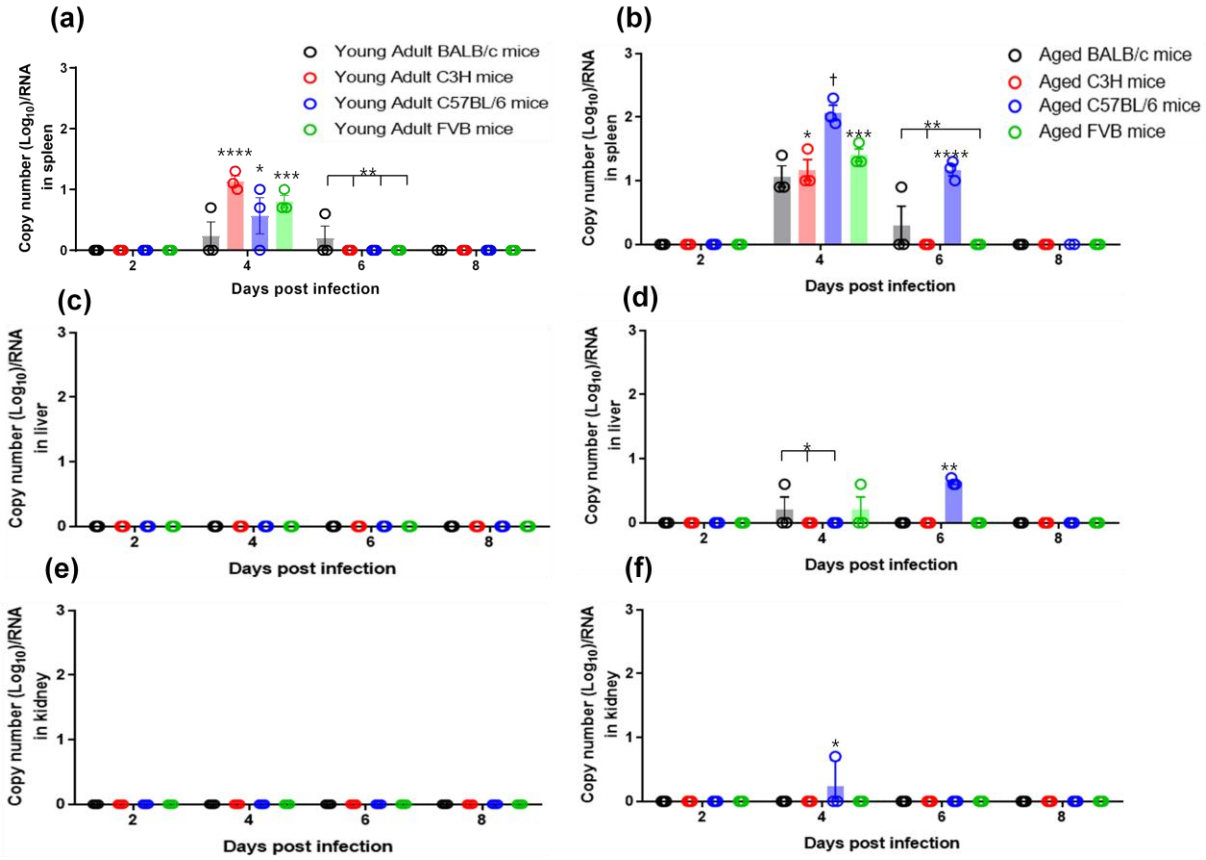
Supplementary Figure 1. Pathogenicity of SFTSV (CB1/2014) in a young adult animal model. Different strains of mice (BALB/c, C3H, C57BL/6, and FVB) were used. Groups of 8-week-old mice (n=5 per group) were intramuscularly inoculated with $10^{6.6}$ $TCID_{50}$ of virus. All groups were observed for morbidity and mortality for 16 days. The percentage of survival (a) and weight loss (b) are shown. Young adult ferrets (n=5, ≤2 years old) were intramuscularly inoculated with $10^{7.6}$ $TCID_{50}$ of virus. Ferrets were observed for morbidity and mortality for 16 days. The percentage of survival (c), weight loss (d), and temperature change (e) are shown.



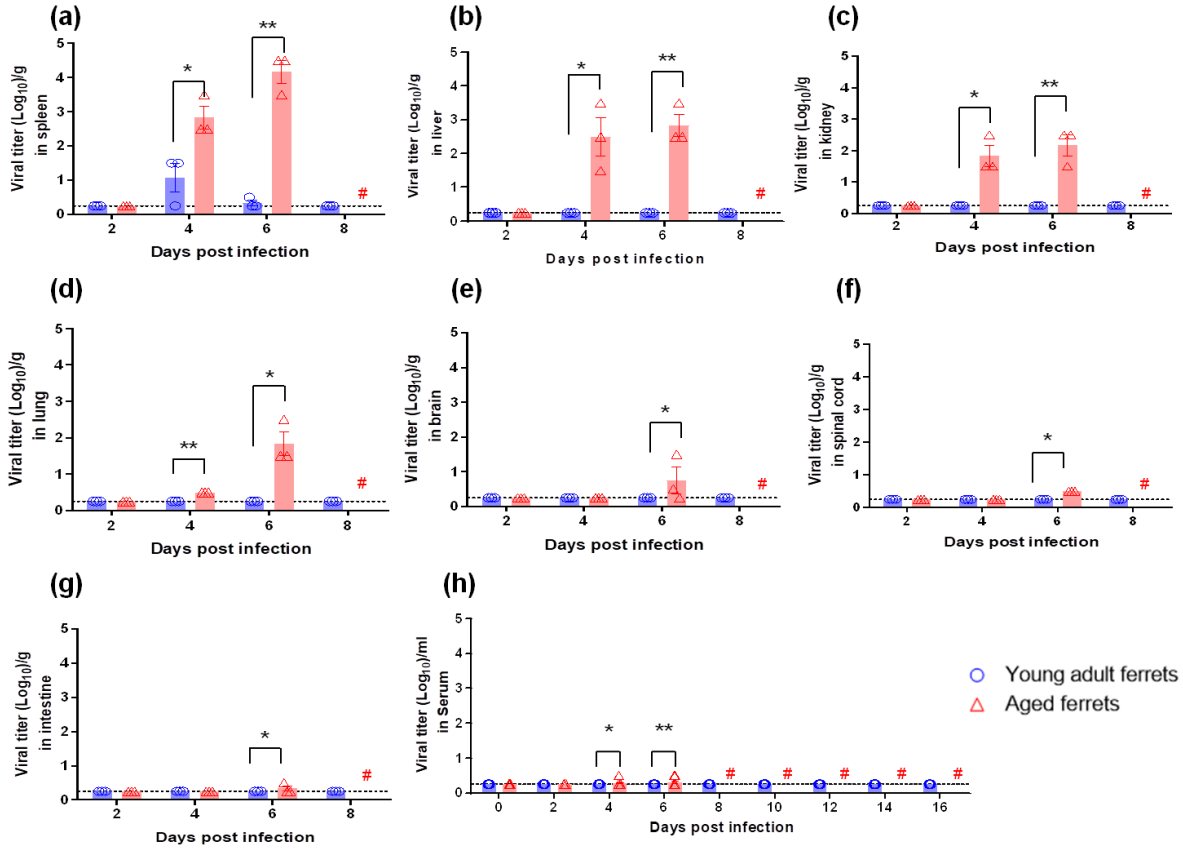
Supplementary Figure 2. Pathogenicity of SFTSV (CB1/2014) in an aged animal model. Different strains of mice (BALB/c, C3H, C57BL/6, and FVB) were used. Groups of mice more than 20 months old (n=5 per group) were intramuscularly inoculated with $10^{6.6}$ $TCID_{50}$ of virus. All groups were observed for morbidity and mortality for 16 days. The percentage of survival (a) and weight loss (b) are shown. Aged ferrets (n=5, ≥4 years old) were intramuscularly inoculated with $10^{7.6}$ $TCID_{50}$ of virus. Ferrets were observed for morbidity and mortality for 16 days. The percentage of survival (c), weight loss (d), and temperature change (e) are shown.



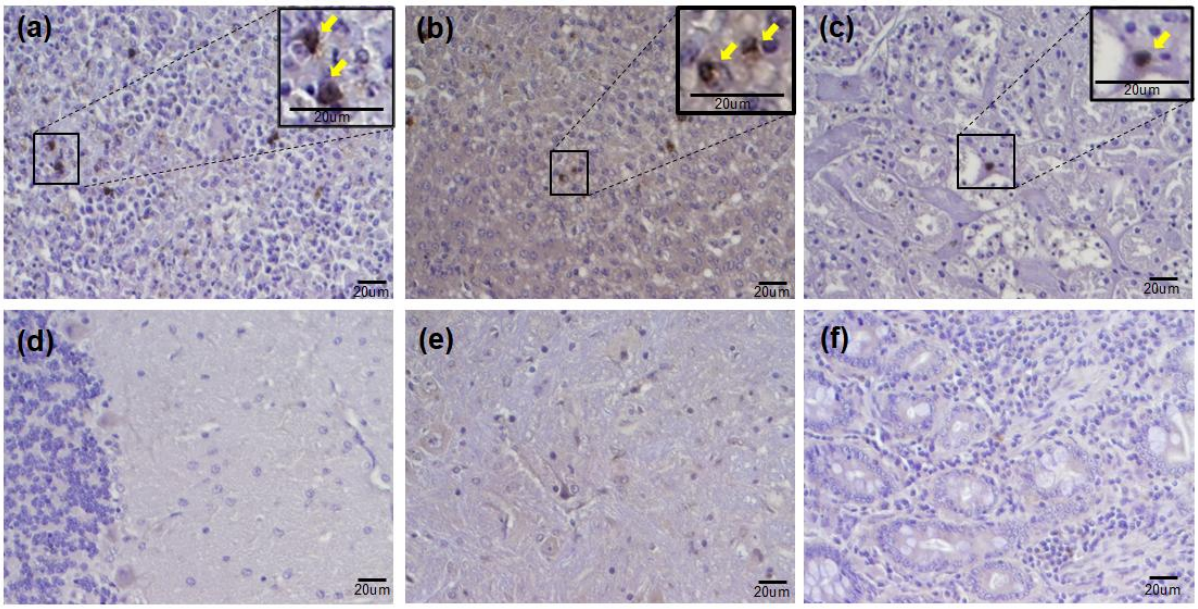
Supplementary Figure 3. Blood analysis of SFTSV-inoculated ferrets (n=21/group). The blood from infected ferrets was collected every other day and hematological examinations were performed using a Celltac hematology analyzer. Red blood cell counts in young adult ferrets (a), red blood cell counts in aged ferrets (b), hemoglobin counts in young adult ferrets (c) and, hemoglobin counts in aged ferret (d). Normal ranges of hemoglobin and RBCs in ferrets are 8.3-18.2 g/dl and 4.8-13.2x10^6/µl, respectively[23,24]. Blue and red represent young and aged ferrets, respectively. The dashed lines are the normal value of red blood cell or hemoglobin. Sharp (#) indicates no samples collected because the ferrets in this group died. Data was presented in box showing upper (75%) and lower (25%) quartiles, with horizontal line as median and whiskers as maximum and minimum values observed. Asterisks indicate statistical significance between non infected- and infected- ferrets per days of post infection as determined by one two tailed unpaired *t*-test. (a) * indicates $p = 0.0095$, ** $p = 0.0042$, *** $p = 0.0005$, **** $p = 0.0003$ and † $p < 0.0001$, (b) * $p = 0.7448$, ** $p = 0.6798$ and *** $p = 0.2552$, (c) * $p = 0.0133$, ** $p = 0.0017$, *** $p = 0.0007$, **** $p = 0.0006$, † $p = 0.0004$ and †† $p < 0.0001$) and, (d) * $p = 0.9199$, ** $p = 0.6187$ and *** $p = 0.4367$.



Supplementary Figure 4. Distribution of the number of viral RNA copies in tissues and blood of SFTSV-infected different strains of mice (BALB/c, C3H, C57BL/6, and FVB). Tissues (n=3/group) and blood samples (all infected mice) were collected at 0, 2, 4, 6 and 8dpi, and tested for viral RNA copies of each specimen of infected mice by real time PCR. The viral copy number of spleen, liver and kidney of each young adult mouse strains (a, c, and e) were compared with those of aged mouse strains (B, D, and f). The black, red, blue and green represent the BALB/c, C3H, C57BL/6, and FVB, respectively. Data (mean ± SEM) was presented with box and dots plots. Asterisks and daggers indicate statistical significance between BALB/c and each strain by the two tailed unpaired *t*-test. (a) * p = 0.4267, ** p = 0.3739, *** p = 0.0894 and **** p = 0.0226, (b) * p = 0.6932, ** p = 0.3739, *** p = 0.1615, **** p = 0.0502, and † p = 0.0082, (d) * p = 0.3739 and ** p < 0.0001, and (f) * p = 0.3739.



Supplementary Figure 5. Distribution of the virus isolation titers in tissues and blood of SFTSV-infected ferrets. Tissues (n=3 per group) were collected at 2, 4, 6, and 8 dpi and blood samples (n=21/group) were collected at 0, 2, 4, 6, 8, 10, 12, 14, or 16 dpi. The virus titers of spleen (a), liver (b), kidney (c), lung (d), brain (e), spinal cord (f), intestine (g), and serum (h) were measured in Vero E6 cells by $TCID_{50}$/g or $TCID_{50}$/ml. Blue and red represent the Young and aged ferrets in each graph, respectively. Sharp (#) indicates no samples collected because the ferrets in this group died. Data (mean ± SEM) was presented with box and dots plots. Asterisks indicate statistical significance compared with each time point sample by the two tailed unpaired *t*-test. (a) * $p = 0.0305$ and ** $p = 0.0004$, (b) * $p = 0.0176$ and ** $p = 0.0015$, (c) * $p = 0.0090$ and ** $p = 0.0045$, (d) * $p = 0.0090$ ** and $p < 0.0001$, (e) * $p = 0.2606$, (f) * $p < 0.0001$, (g) * $p = 0.3739$, and (h) * $p = 0.1326$ and ** $p = 0.0349$.



Supplementary Figure 6. Immunohistochemistry of tissues from young adult ferrets infected with SFTSV. Ferrets were inoculated intramuscularly with $10^{7.6}$ $TCID_{50}$. Tissues were harvested on day 6 after inoculation. Spleen (a), liver (b), kidney (c), brain (d), spinal cord (e), and intestine (f). Presence of SFTSV NP antigen was determined by IHC. Magnification x 400 (scale bar = 20μm). SFTSV positive regions were magnified x 400 (scale bar = 20μm) of each figure. Experiments were performed with three independent trials and all results were reproduced.

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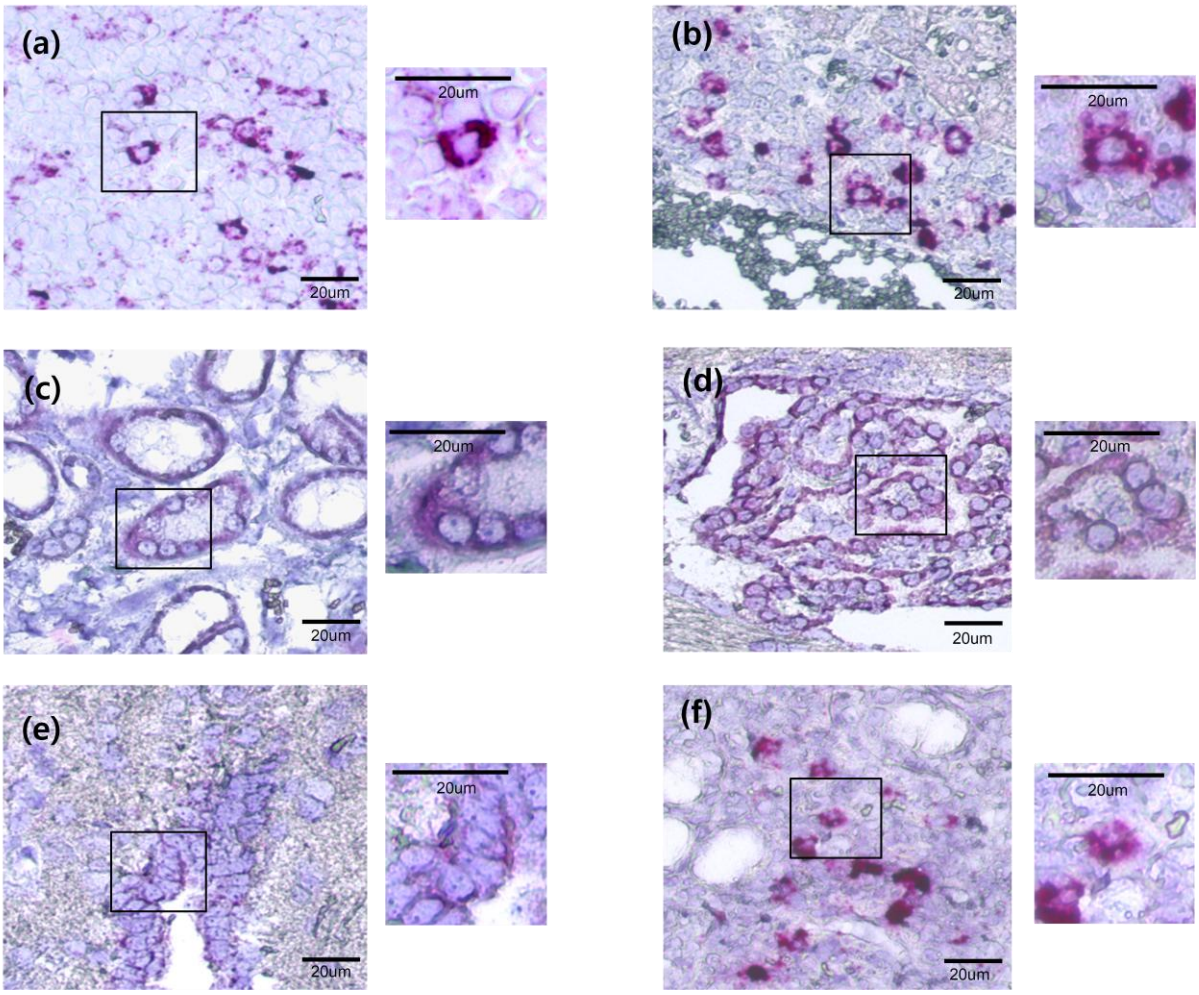
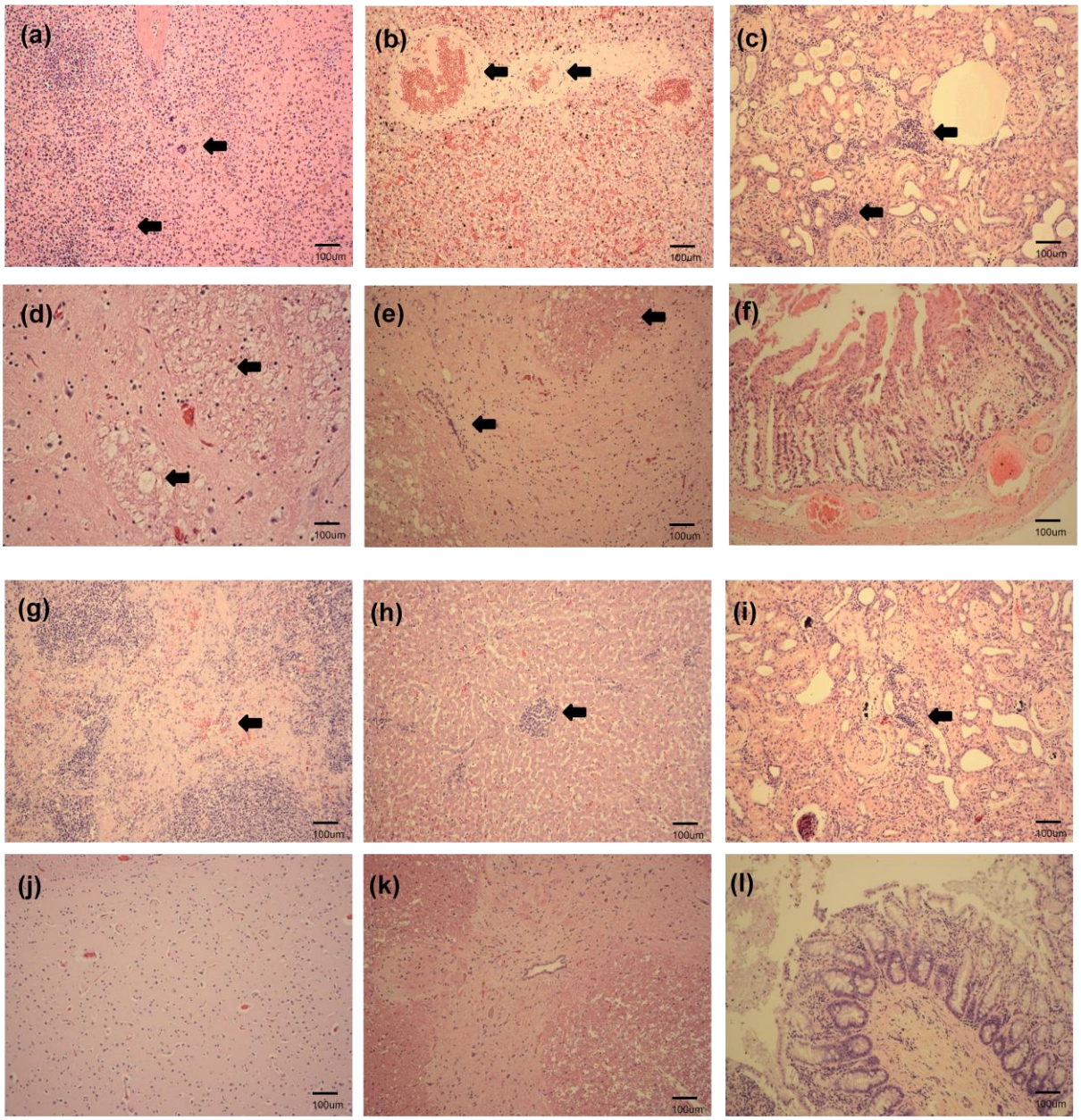
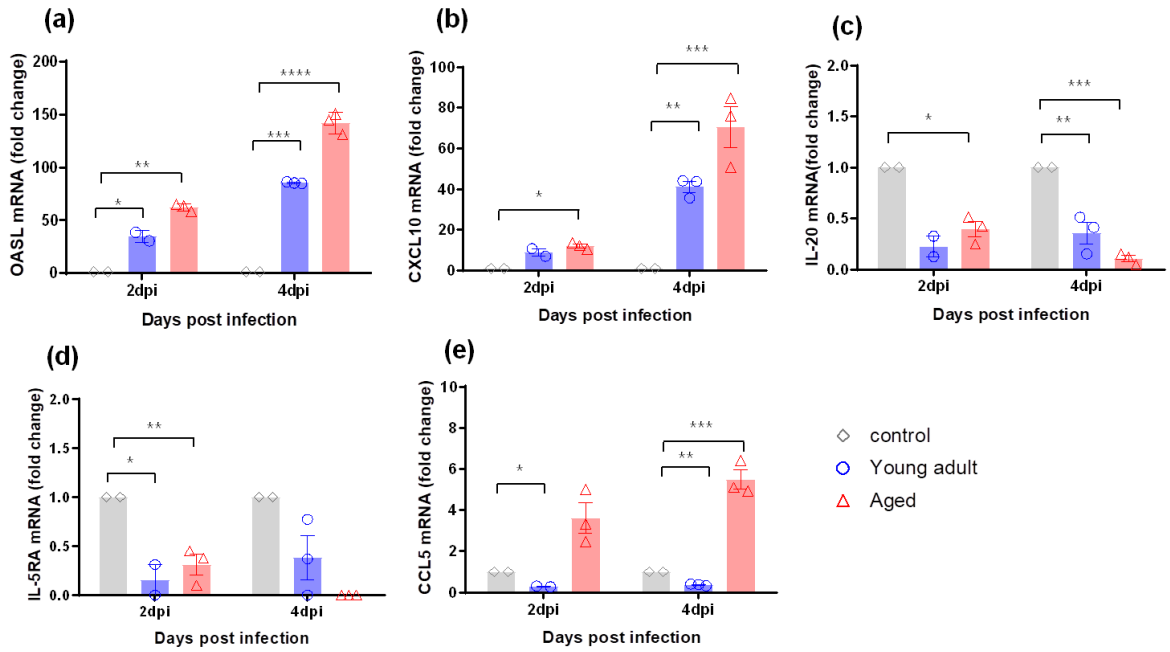


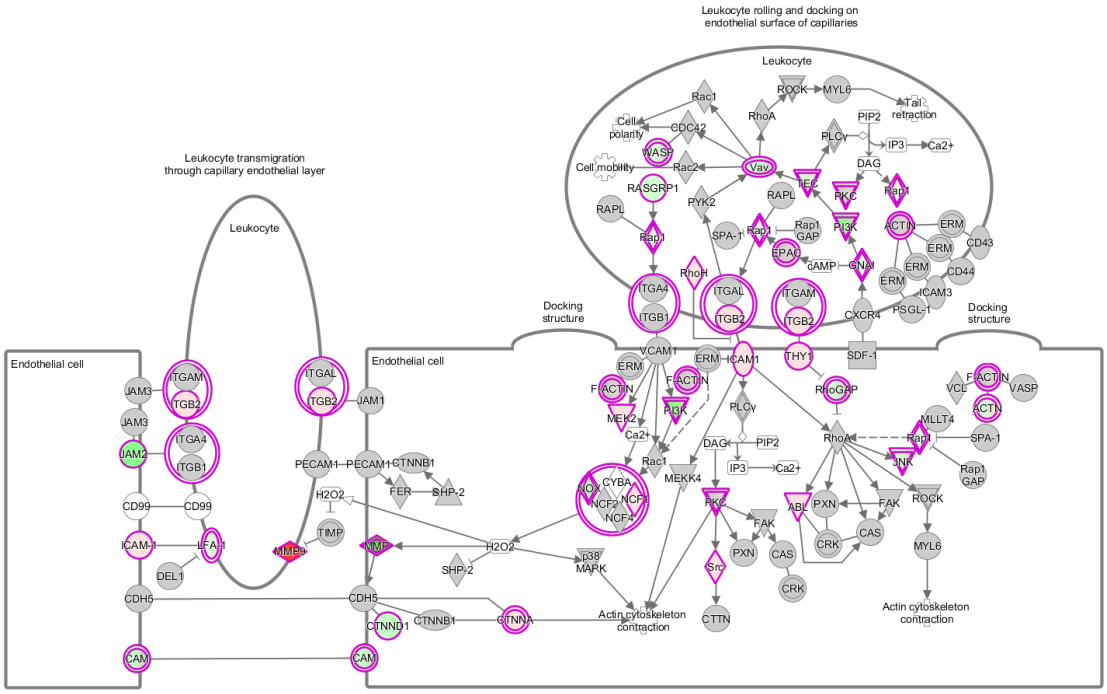
Supplementary Figure 7. Detection of SFTSV RNA using RNAscope in situ hybridization. SFTSV-infected tissues were harvested at 6 dpi from aged ferrets and tissues were labeled with SFTSV-NP specific probes. Magnification x 200 (scale bar = 20μm). SFTSV NP RNA positive regions were magnified x 400 (scale bar = 20μm) of each figure: Spleen (a), liver (b), kidney (c), brain (d), spinal cord (e), and intestine (f). Experiments were performed with three independent trials and all results were reproduced.



Supplementary Figure 8. H&E staining of organs from SFTSV-infected aged (a-f) and young adult ferrets (g-l). Ferrets were inoculated intramuscularly with $10^{7.6}$ $TCID_{50}$. Tissues were harvested on day 6 after inoculation. Spleen (a, g), liver (b, h), kidney (c, i), brain (d, j), spinal cord (e, k), and intestine (f, l). The SFTSV-infected aged ferret tissues showed highly inflamed compared with those of young adult ferrets. Arrows indicate the inflamed regions in each tissue. Magnification x 100 (scale bar = 100μm). Experiments were performed with three independent trials and all results were reproduced.



Supplementary Figure 9. Differently expressed genes in PBMC from young adult and aged ferrets. Overview of RNA sequencing data from PBMCs of young adult and aged ferrets infected with SFTSV and control ferrets at 2 and 4 dpi (n=2 for control, and 2dpi of young adult ferrets, and n=3 for 4dpi of young adult ferrets and 2dpi and 4dpi of aged ferrets). Expression of genes OASL (a), CXCL10 (b), IL-20 (c), IL5RA (d), and CCL5 (e), in PBMCs were normalized to GAPDH and expressed as fold changes relative to non-infected controls. Data (mean ± SEM) was presented with box and dots plots. Asterisks indicate statistical significance compared with each dpi sample by the two tailed unpaired *t*-test (a) * $p = 0.0143$, ** $p = 0.0001$, *** $p < 0.0001$, and *** $p = 0.0003$, (b) * $p = 0.0023$, ** $p = 0.0016$, and, *** $p = 0.0134$, (c) * $p = 0.0086$, ** $p = 0.163$ and *** $p = 0.0004$, (d) * $p = 0.0391$, and ** $p = 0.0141$, and (e) * $p = 0.0047$, ** $p = 0.0007$, and *** $p = 0.0054$. Experiments were performed with three independent trials and similar results were reproduced.



Supplementary Figure 10. Analysis of leukocyte extravasation signaling at 2 dpi. To identify pathways that were differentially expressed between infected aged and young adult ferrets at 2 dpi, pathway analysis was done using the IPA software. Genes associated with leukocyte extravasation were differentially expressed as shown by the highlighted color. Color indicates the degree of upregulation (red) or downregulation (green) of genes relative to young adult infected ferrets. Solid lines represent direct interactions and dashed lines indirect interactions.