Supporting Information

Souza et al. 10.1073/pnas.0906467106

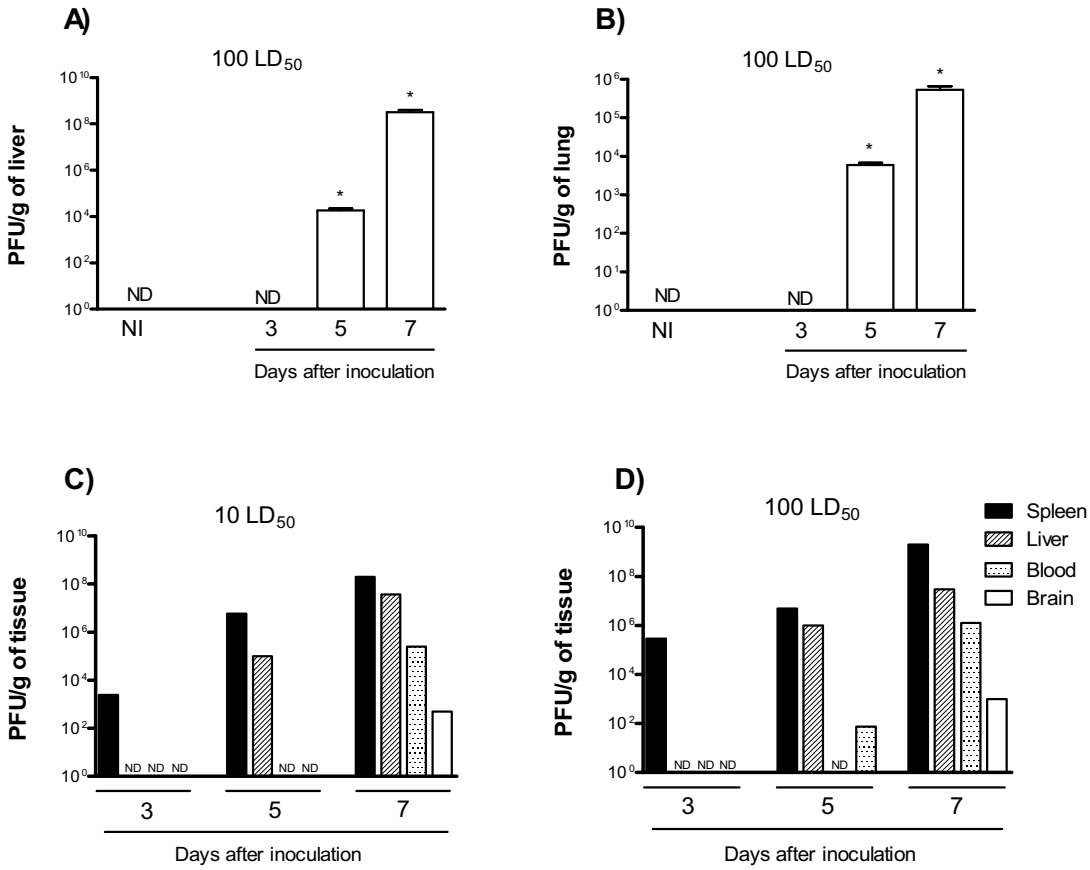

Fig. S1. Viral loads in liver, spleen, blood, and brains of BALB/c mice after inoculation with an adapted DEN-2 strain. In (*A*) and (*B*), mice were inoculated with 100 LD_{50} and the viral loads of DEN-2 were evaluated using a plaque assay in liver (*A*) and in lungs (*B*) at days 3, 5, and 7 after inoculation. Results are the mean ± SEM and there were 6 animals in each experimental group and are expressed as PFU/g tissue. In (*C*) and (*D*), mice were inoculated with 10 LD_{50} (*C*) or 100 LD_{50} (*D*) of the adapted-DEN-2 and the viral loads were evaluated 3, 5, and 7 days after infection by a plaque assay. Results is a representative value found in 1 of 2 assays of pooled tissue homogenates of 6 animals in each group and are expressed as PFU/g of tissue. NI, non-infected mice. ND, not detected.

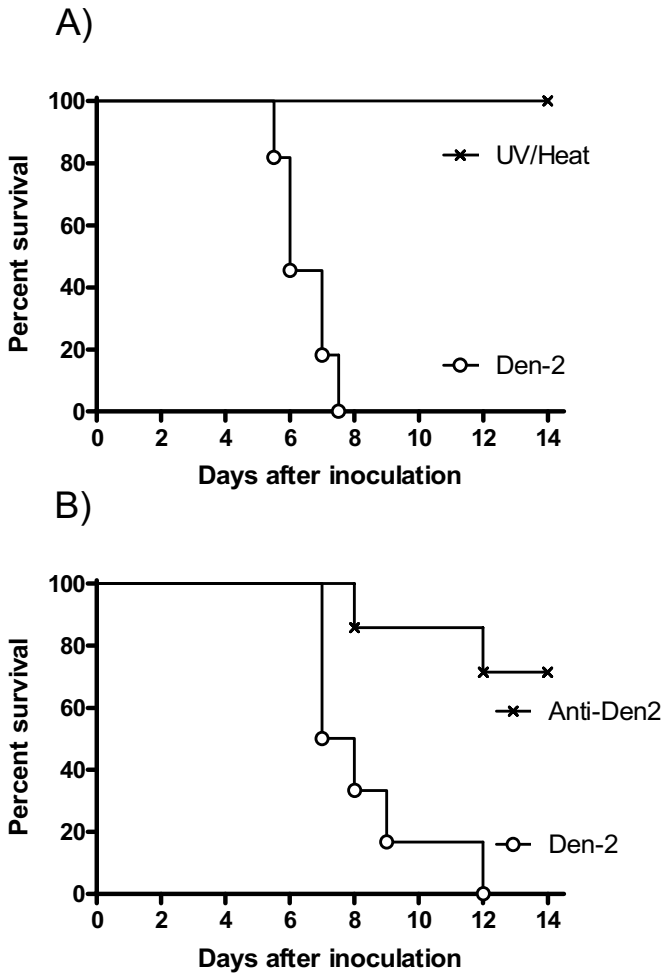

Fig. S2. Lethality of DEN-2-inoculated BALB/c mice is prevented by heat or UV-inactivation or by pretreatment with an anti-DEN-2 antiserum. In (*A*), the viral inoculum (100 LD_{50}) was heat inactivated (Heat, 56 °C, 60 min) or treated with UV light (UV, 7 min) before inoculation of BALB/c mice. In (*B*), animals were pretreated i.p. with 100 μL anti-DEN-2 antiserum or control serum (preimmune serum) 60 min before inoculation with 100 LD_{50} of DEN-2. Lethality was evaluated every 12 h ($n = 8$ mice per group). There was significant protection ($P < 0.01$) of mice inoculated with inactivated virus or treated with the anti-DEN-2 antiserum.

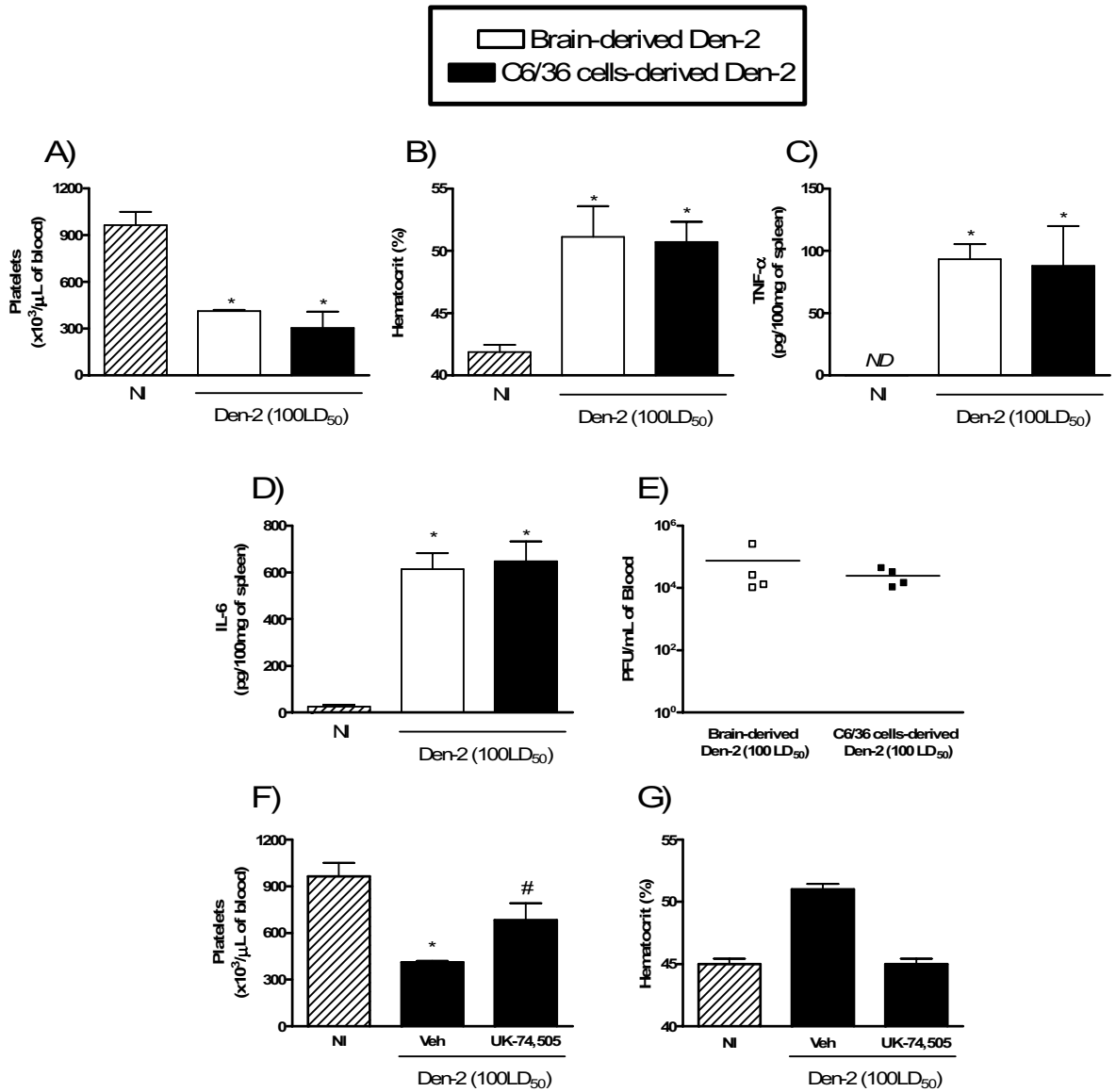

Fig. S3. Passage of adapted-Den-2 virus in permissive cell cultures do not alter the pathological findings induced by virus inoculation in BALB/c mice. Mice were inoculated with 100 LD_{50} of brain-derived Den-2 (white bars) or C6/36 cell-derived Den-2 (black bars). Control non-infected mice (NI) were injected with brain suspension from normal animals. On day 7, mice were culled and several parameters of the infection determined. The number of platelets (*A*) and hematocrit (*B*) in blood of NI and DEN-2-infected mice were evaluated as described in the *Methods* section. Platelet counts are shown as the number of platelets $\times 10^3/\mu$L blood and hematocrit as % volume occupied by red blood cells. The levels of TNF-α (*C*) and IL-6 (*D*) in spleen were determined by ELISA and are shown as pg cytokine per 100 mg spleen. Results are the mean $\pm$ SEM, and there were 6 animals in each experimental group. In (*E*), viral loads in blood of individual mice at day 7 after infection. In (*F*) and (*G*), note the effects of the treatment with the PAFR antagonist, UK-74,505, in mice infected with the C6/36-derived Den-2. UK-74,505 was given at the dose of 10 mg/kg twice a day and treatments was started on day 5 and continued until day 7 after inoculation. *, $P < 0.01$ when comparing Den-2 infected with NI mice and # for $P < 0.01$ when comparing to UK-74,505-treated and infected mice. NI, non-infected mice. ND, not detected.

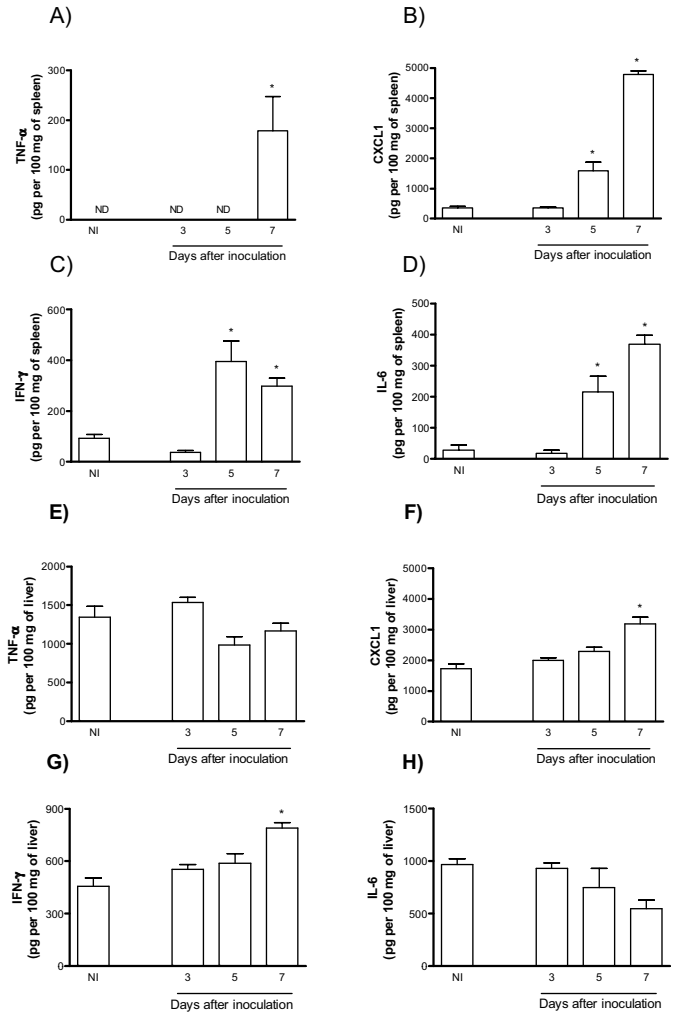

Fig. S4. Levels of cytokines in the *spleen and liver* of BALB/c mice after inoculation with an adapted DEN-2 strain. Mice were inoculated with 100 LD_{50} and the concentrations of TNF-a (*A* and *E*), CXCL1 (*B* and *F*), IFN-g (*C* and *G*), and IL-6 (*D* and *H*) in the spleen and liver evaluated by ELISA at days 3, 5, and 7 after inoculation. Control non-infected mice (NI) were injected with brain suspension from normal animals. Results are shown as pg cytokine per 100 mg spleen or liver and are the mean ± SEM of $n = 6$ animals in each group. **, $P < 0.01$ when compared to control uninfected mice. ND, not detected.

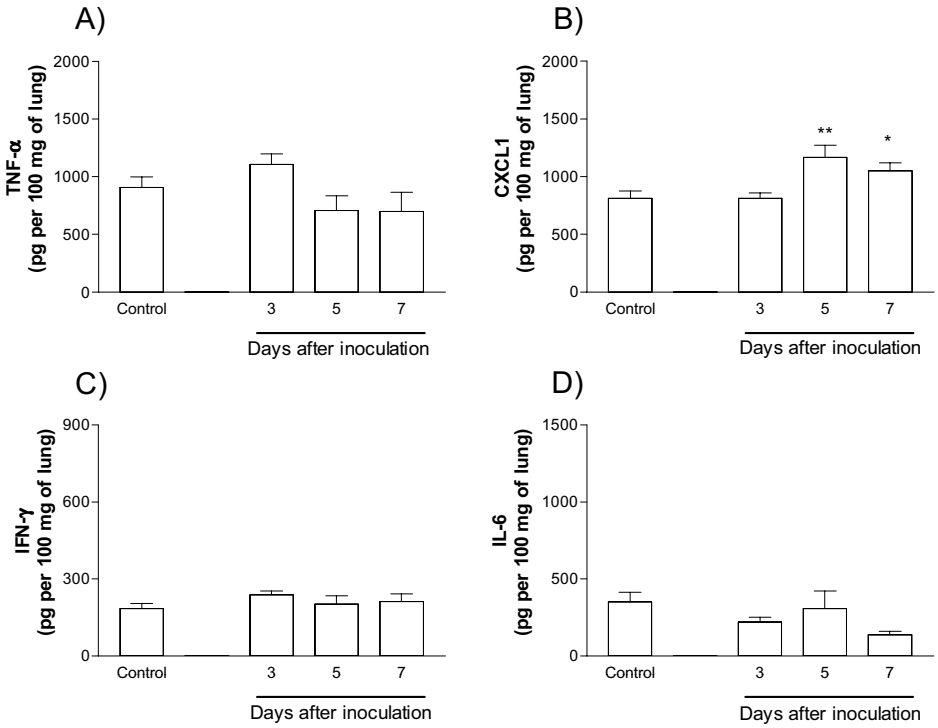

Fig. S5. Levels of cytokines in the *lungs* of BALB/c mice after inoculation with an adapted DEN-2 strain. Mice were inoculated with 100 LD_{50} and the concentrations of TNF-α (*A*), CXCL1 (*B*), IFN-γ (*C*), and IL-6 (*D*) in the lungs evaluated by ELISA at days 3, 5, and 7 after inoculation. Control non-infected mice (NI) were injected with brain suspension from normal animals. Results are shown as pg cytokine per 100 mg lungs and are the mean $\pm$ SEM of $n = 6$ animals in each group. **, $P < 0.01$ when compared to control uninfected mice. ND, not detected.

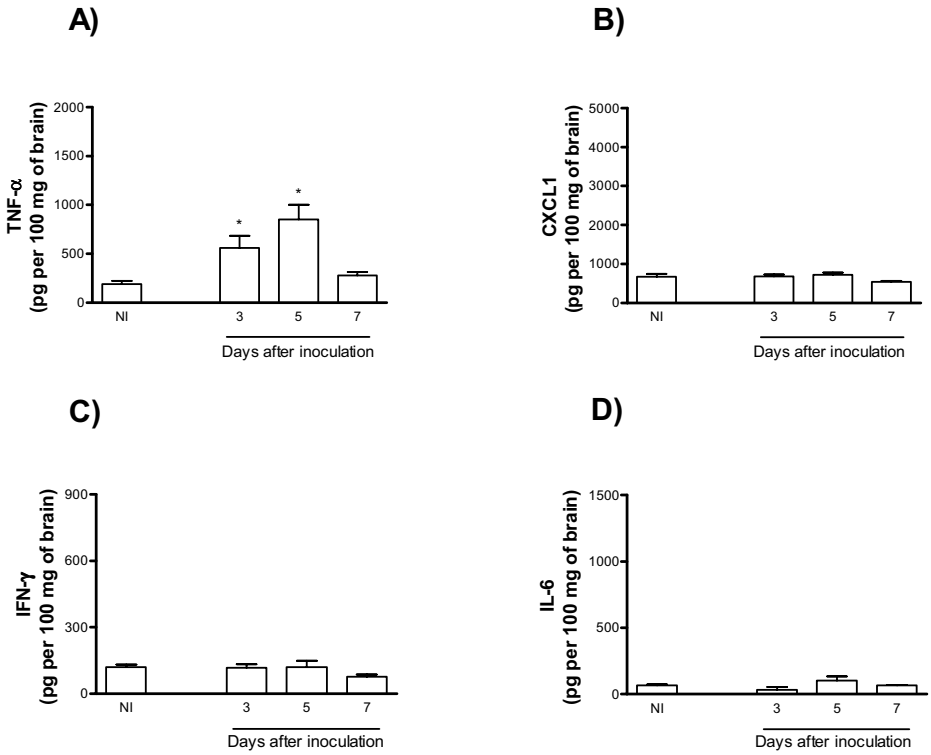

Fig. S6. Levels of cytokines in the *brain* of BALB/c mice after inoculation with an adapted DEN-2 strain. Mice were inoculated with 100 LD_{50} and the concentrations of TNF-α (*A*), CXCL1 (*B*), IFN-γ (*C*), and IL-6 (*D*) in the brain evaluated by ELISA at days 3, 5, and 7 after inoculation. Control non-infected mice (NI) were injected with brain suspension from normal animals. Results are shown as pg cytokine per 100 mg brain and are the mean $\pm$ SEM of $n = 6$ animals in each group. **, $P < 0.01$ when compared to control uninfected mice. ND, not detected.

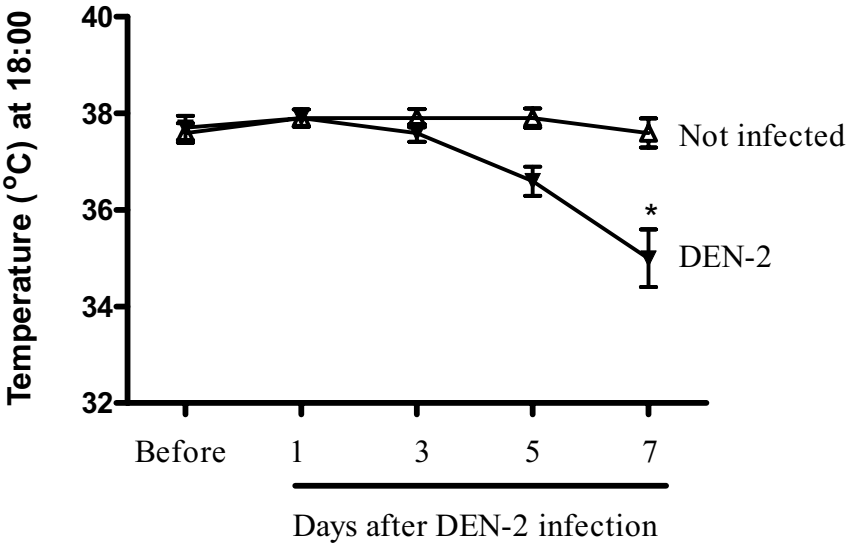

Fig. S7. Body temperature of BALB/c mice after inoculation with an adapted DEN-2 strain. Mice were inoculated with 100 LD_{50} and the body temperature was monitored each 2 days by a probe installed in the animal abdomen. Control non-infected mice (NI) were injected with brain suspension from normal animals. Results are shown as °C and are the mean ± SEM of $n = 6$ animals in each group. **, $P < 0.01$ when compared to control uninfected mice.

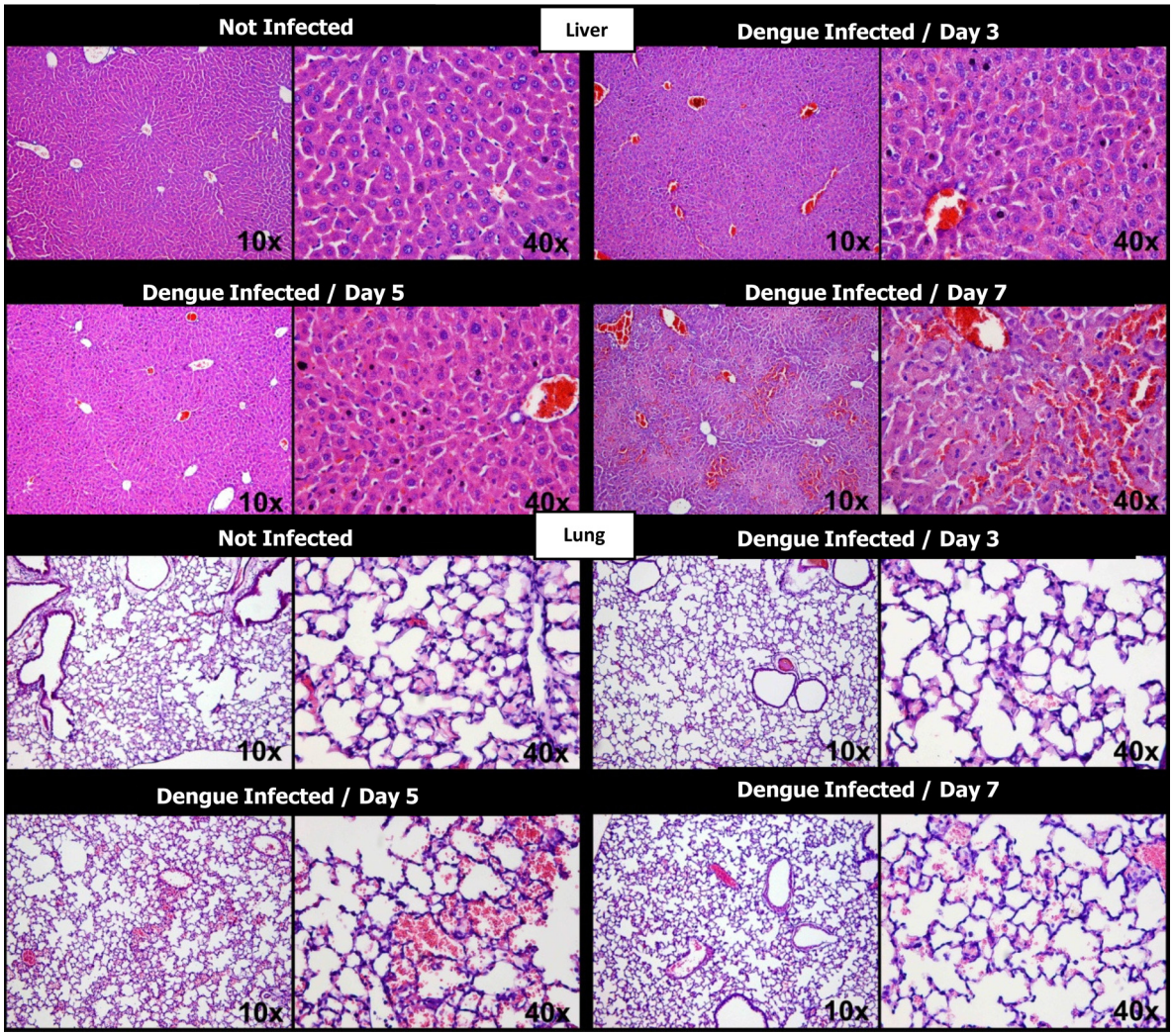

Fig. S8. Histopathological evaluation of the liver and lungs of BALB/c mice after inoculation with an adapted DEN-2 strain. Mice were inoculated with 100 LD_{50} and the liver and lungs obtained at days 3, 5, and 7 after inoculation. Control non-infected mice (Not Infected) were injected with brain suspension from normal animals. The organs were processed for H&E staining. The photomicrographs were taken from representative sections of at least 4 animals in each group (100 or 400×).

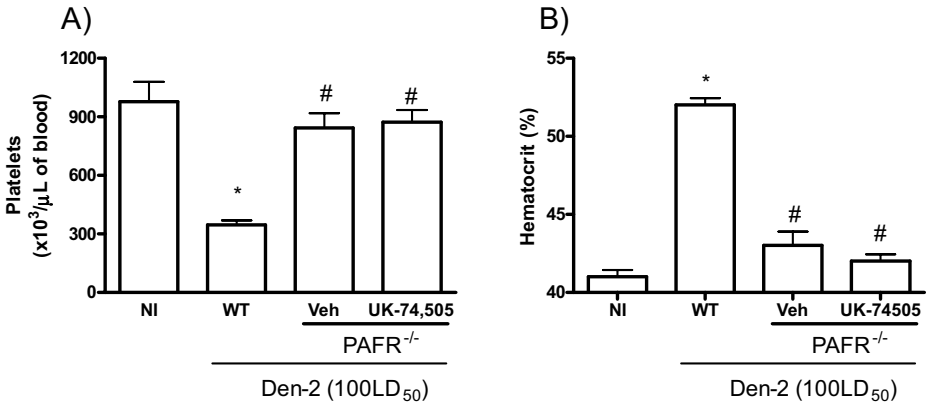

Fig. S9. Effects of the administration of the PAFR antagonist UK-74,505 to PAFR-deficient mice. WT or PAFR-deficient mice (PAFR-/-) were inoculated with 100 LD_{50} of brain-derived Den-2. Control non-infected mice (NI) were injected with brain suspension from normal animals. On day 7, mice were culled and the number of platelets (*A*) and hematocrit (*B*) in blood evaluated as described in the *Methods* section. Platelet counts are shown as the number of platelets x $10^3/\mu$L blood and hematocrit as % volume occupied by red blood cells. UK-74,505 was given at the dose of 10 mg/kg twice a day and treatments was started on day 5 and continued until day 7 after inoculation. *, $P < 0.01$ when comparing Den-2 infected with NI mice and # for $P < 0.01$ when comparing to PAFR-deficient and WT mice.

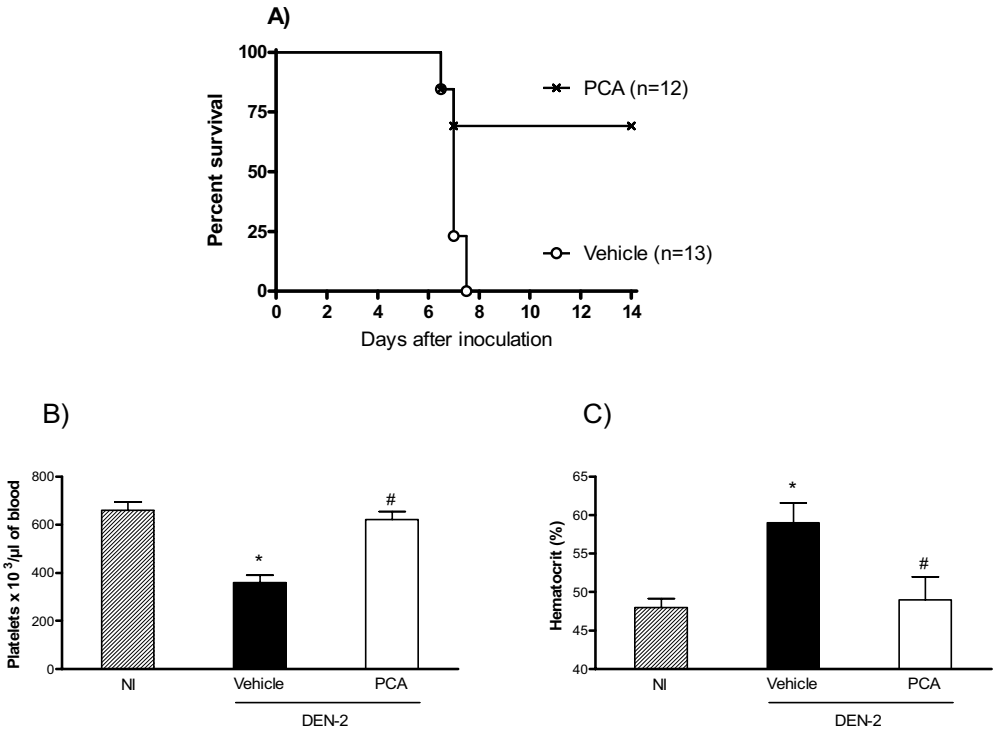

Fig. S10. Lethality is delayed and disease is prevented by treatment of DEN-2-inoculated BALB/c mice with the PAFR antagonist PCA-4248. Vehicle or PCA-4248-treated mice were inoculated with 100 LD_{50} of DEN-2 and lethality (*A*) was evaluated every 12 h ($n = 8$ mice per group). The number of platelets (*A*) and hematocrit (*B*) in blood of NI and of Vehicle or PCA-4248-treated DEN-2 infected mice were evaluated 7 days later, as described in the *Methods* section. Platelet counts are shown as the number of platelets $\times 10^3/\mu$L blood and hematocrit as % volume occupied by red blood cells. PCA-4248 was given at the dose of 5 mg/kg twice a day and treatments was started on day 5 and continued until day 10 after inoculation. There was significant protection ($P < 0.01$) of mice treated with PCA-4248.