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Supplementary Materials for

Arenaviral infection causes bleeding in mice due to reduced serotonin release from platelets

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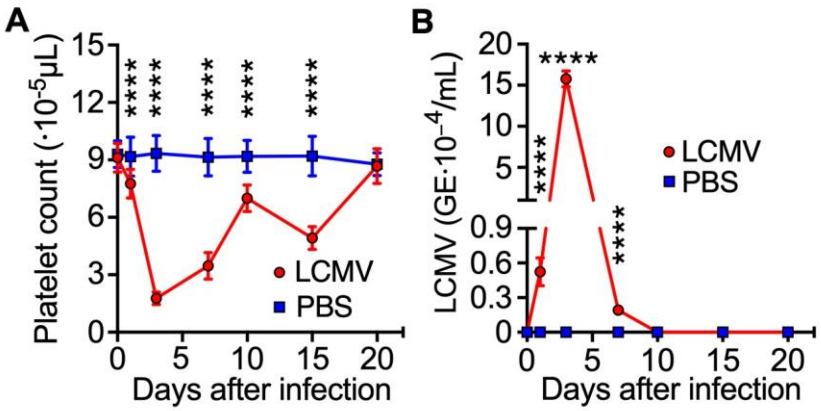
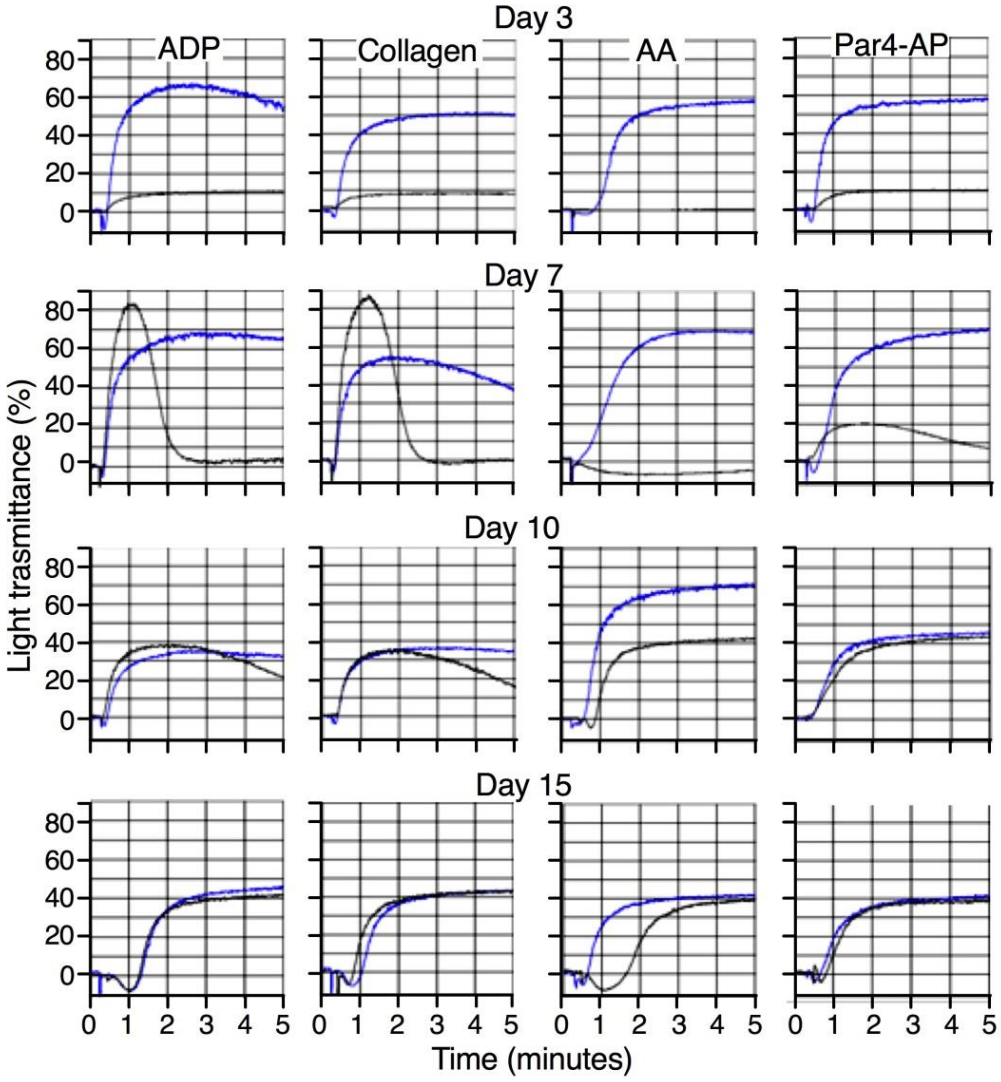
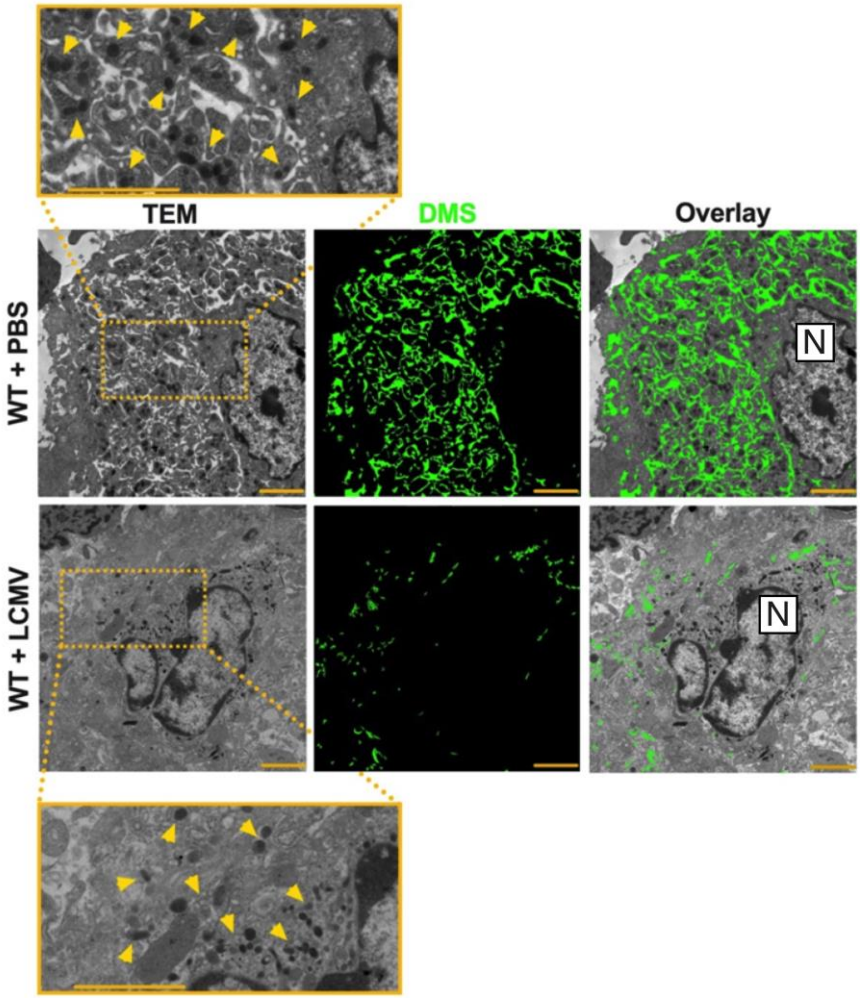


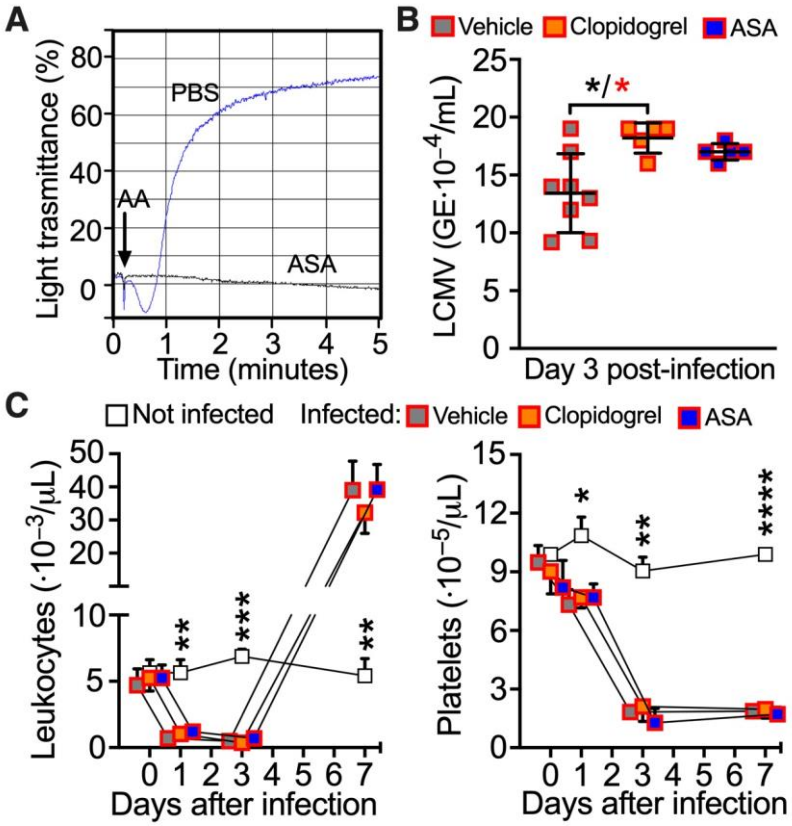
Supplementary Figure 1. Blood platelet count and viral genome content after LCMV infection. WT mice were injected with $5 \cdot 10^5$ pfu LCMV Arm (red circles) or control PBS (blue squares). Blood samples were taken at different time points after treatment to count platelets (10 control and 35 infected mice) (**A**) and to measure LCMV Arm genome equivalent (GE; 4 control and 9 infected mice) (**B**). Data shown as mean ±SD were analyzed by repeated measures two-way ANOVA with the Geisser-Greenhouse correction for no assumption of sphericity and Šidák's post-test for multiple paired comparisons at different time points. ****P<0.0001.



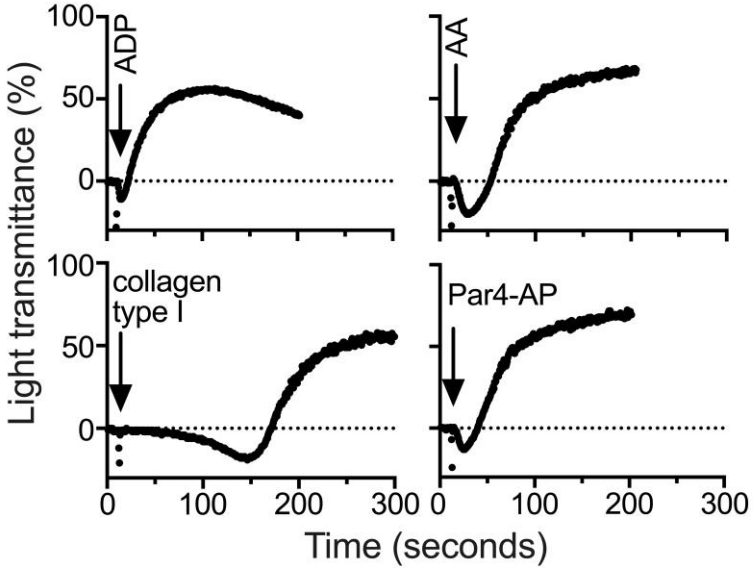
Supplementary Figure 2. Representative traces of platelet aggregation at different time points after LCMV infection. Platelets in PRP of WT mice infected with LCMV (black traces) or injected with PBS as control (blue traces) were stimulated with 5 μM ADP, 4 μg/ml collagen, 75 μM arachidonic acid (AA) or 40 μM protease-activated receptor 4 activation peptide (Par4-AP). Blood samples (n =10 mice for each condition) were collected at the indicated days after infection for testing.



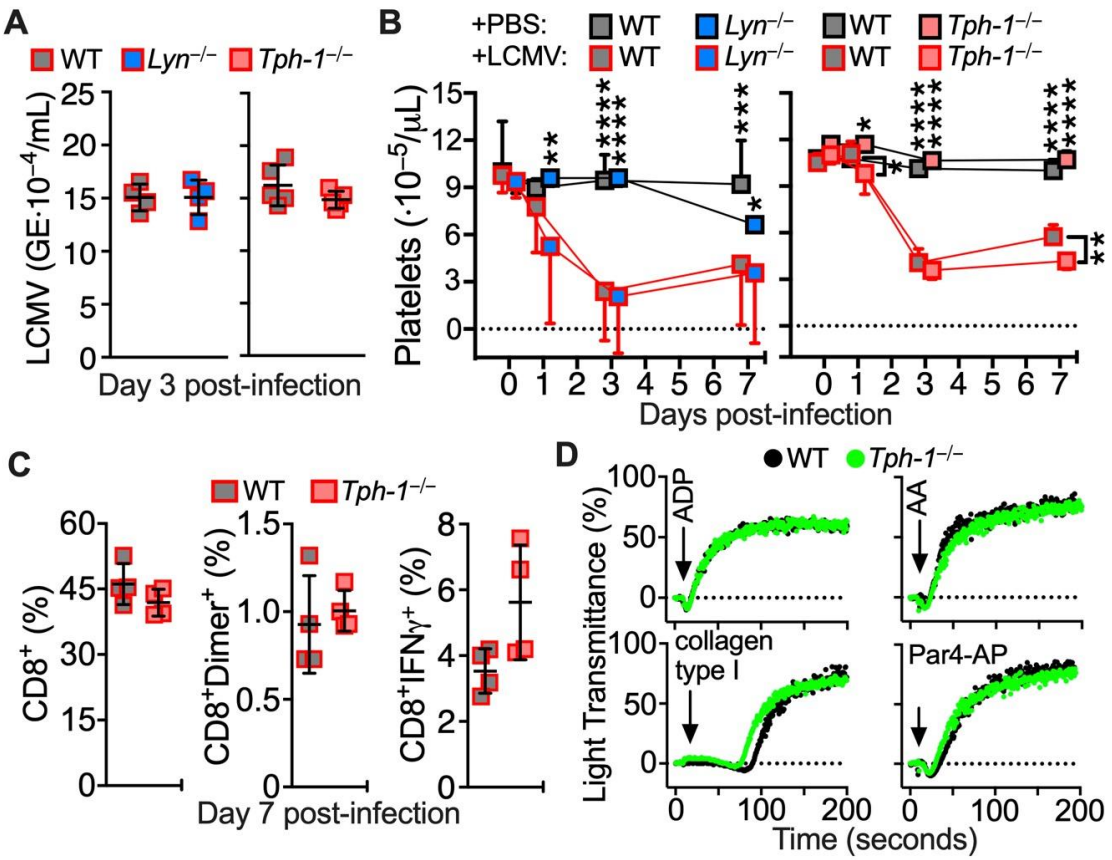
Supplementary Figure 3. Transmission electron microscopy (TEM) of megakaryocytes after LCMV infection. Representative micrographs of megakaryocytes isolated from the bone marrow of WT mice infected with $5 \cdot 10^5$ pfu LCMV Arm or injected with PBS as control. Left: The megakaryocytes of infected mice (WT+LCMV) compared to controls (WT+PBS) show a poorly delineated demarcation membrane system (DMS) and a reduced number of storage granules localized predominantly in close proximity to the nucleus. The magnified highlighted areas allow a clearer visualization of the granules, of which some clusters are highlighted by yellow arrowheads. Middle: Selective visualization of the DMS using a green mask highlights the difference between infected and control mice. Right: Overlay of the corresponding left and middle panels. N = Nucleus. Scale bar (in orange) =2 µm. (n = 41-85 platelet sections, isolated from 3 mice per group).



Supplementary Figure 4. Viral replication and blood counts of leukocytes and platelets in LCMV-infected mice treated with aspirin or clopidogrel. (**A**) Arachidonic acid-induced aggregation (AA, 75 μM) of platelet-rich plasma from WT mice treated with aspirin (ASA, 10 mg/kg/day intraperitoneally; black trace) or ASA vehicle (PBS; blue trace). (**B**) LCMV Arm genome equivalent (GE) measured 3 days after infection in blood of WT mice treated with ASA (n =5); or clopidogrel, 60 μg/day in drinking water (n =5); or ASA vehicle (n =8). (**C**) Blood counts of leukocytes (left) and platelets (right) in untreated WT mice injected with PBS (not infected; n =4, left and n =3, right) compared to mice treated with ASA or clopidogrel or ASA vehicle from two days before LCMV infection (for all groups, n =4, left and n=5, right). Data in (B) and (C) are shown as scatter plots with mean ± SD of values measured at the indicated time points. Statistical analysis in (B) was performed by Brown-Forsythe ANOVA with Welch's correction and Dunnett's T3 test for multiple comparisons; and by non-parametric Kruskal-Wallis test followed by Dunn's test for multiple comparisons (black and red asterisk, respectively). Data in (C) were analyzed by repeated measures two-way ANOVA with the Geisser-Greenhouse correction for no assumption of sphericity and Dunnett's post-test for multiple paired comparisons of infected vs. non infected groups (significance indicated by asterisks); infected mice treated with aspirin or clopidogrel were not significantly different from those treated with vehicle control. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.



Supplementary Figure 5. Functionality of mouse platelets used for transfusion. Blood collected from WT mice was pooled and platelets were washed free of plasma and other blood cells by sequential centrifugation and resuspension in buffer. The response to agonist-induced activation was evaluated with 5 μM ADP, 4 μg/ml collagen, 75 μM arachidonic acid and 40 μM protease-activated receptor 4 activation peptide (Par4-AP). Representative traces of platelet aggregation are shown; note the delayed response to collagen compared to fig. S6D, which was likely a consequence of the procedure to isolate washed platelets, but in the context of an overall good response to agonist-induced activation.



Supplementary Figure 6. Parameters of LCMV infection and baseline platelet aggregation in WT and mutant mice. (A) Comparison of viral genome equivalent (GE) in blood 3 days after LCMV infection in WT/*Lyn*$^{-/-}$ (n =4; left) and WT/*Tph-1*$^{-/-}$ mice (n =5; right). (**B**) Comparison of platelet count variations after injection of LCMV Arm or control PBS in WT/*Lyn*$^{-/-}$ (left; WT n=15 infected, 10 noninfected, *Lyn*$^{-/-}$ n=20 infected, 10 noninfected) and WT/*Tph-1*$^{-/-}$ mice (right; WT and *Tph-1*$^{-/-}$ n=6 infected, 3 noninfected). (**C**) CD8^{+} (left), CD8^{+}Dimer^{+} (middle) and CD8^{+}IFNγ^{+} T-lymphocytes (the latter induced by virus-specific H2b-restricted peptide GP33-41) measured as percentage of mononuclear cells isolated from peripheral blood of WT and *Tph-1*$^{-/-}$ mice 7 days after injection of LCMV Arm (n =4 per group). Data, shown as mean ± SD, were analyzed by two-tailed Mann-Whitney test (A, C; no significant difference) or repeated measures two-way ANOVA with Tukey's post-test for multiple PBS compared to LCMV comparisons (B); *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001. Asterisks on top of symbols refer to comparisons before/after infection in the same strain; asterisks on the side of symbols refer to comparisons after infection between strains. (**D**) Representative platelet aggregation traces in PRP from untreated WT (black) or *Tph-1*$^{-/-}$ (green) mice stimulated with 5 μM ADP, 75 μM arachidonic acid (AA), 4 μg/ml collagen or 40 μM protease-activated receptor 4 activation peptide (Par4-AP); n =4 mice per group.