

## Supplementary information

### Simulation of hemorrhage pathogenesis in mice through dual stimulation with dengue envelope protein domain III-coated nanoparticles and antiplatelet antibody

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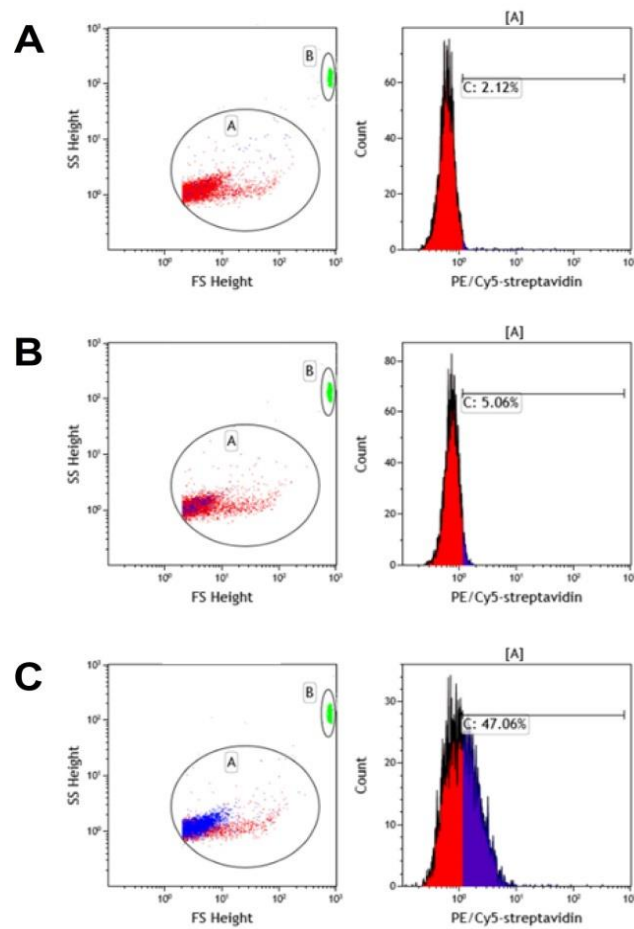
**Key words:** dengue virus; dengue envelope protein domain III; dengue hemorrhage fever; hemorrhage; anti-platelet antibody; silica nanoparticles; inflammation; cytokines; anti-coagulants; two-hit model

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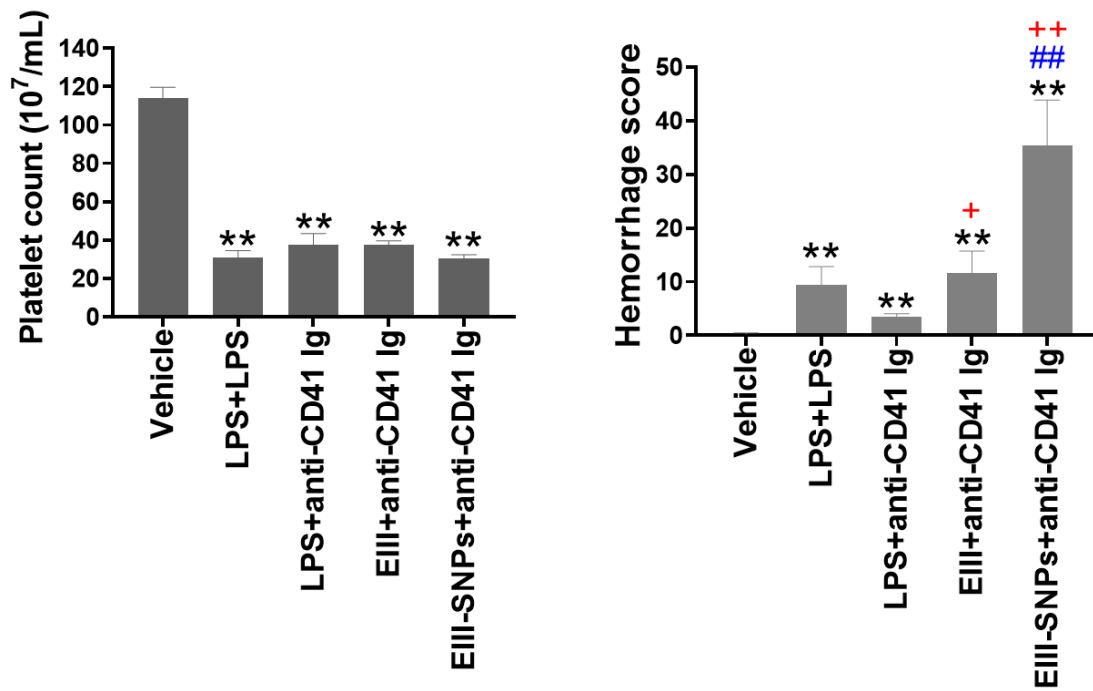
**Figures S1-S4:** pages 2-5

**Figure S1**



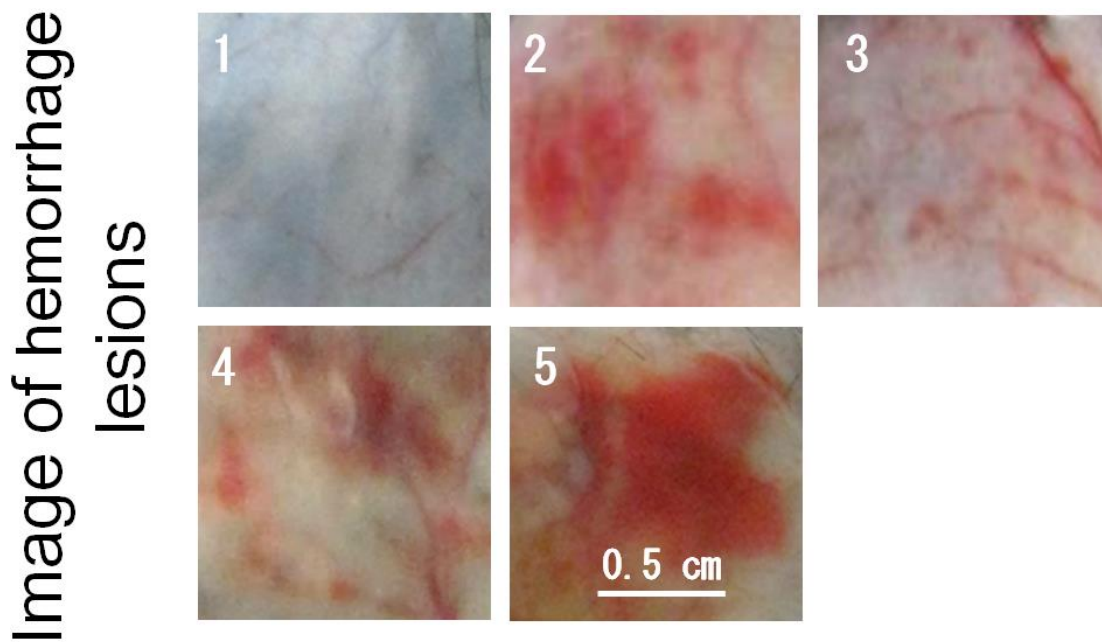
**Figure S1.** The detection of EIII coating on the SNPs was accomplished by measuring the streptavidin-phycoerhthrin-Cy5 (PE/Cy5) binding signal to biotinylated EIII on the SNPs using flow cytometry. Examples of flow cytometry gating graphs of (A) SNPs only (without staining) groups, (B) SNPs + streptavidin-PE/Cy5 groups, and (C) EIII-SNPs + streptavidin-PE/Cy5 groups.

Figure S2



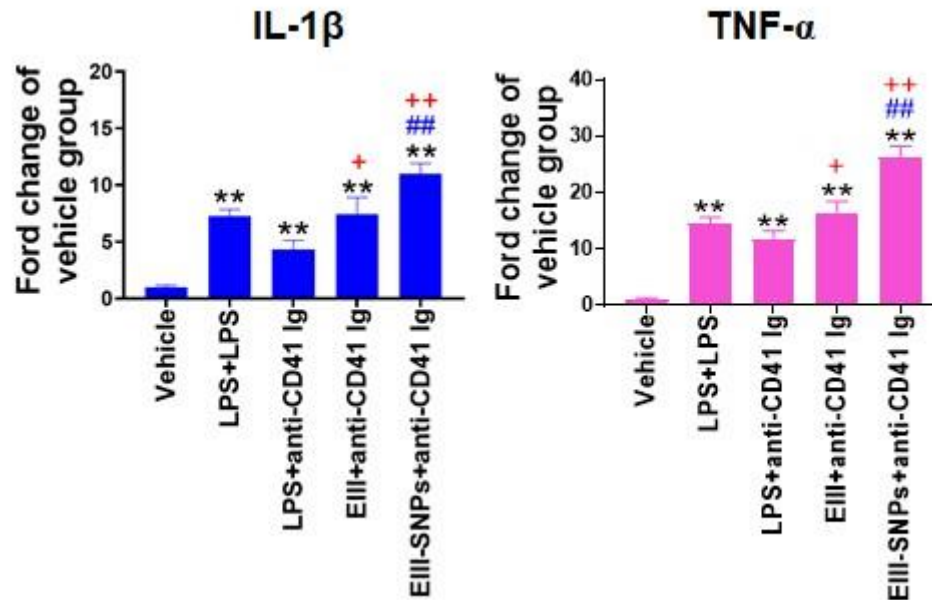
**Figure S2. Comparisons of platelet counts and hemorrhage score after different two-hit treatments in mice.** Platelet counts and hemorrhage score were analyzed in mice groups without (vehicle), and with different 2-hit treatments [LPS (200  $\mu$ g/kg) + LPS (1.2 mg/kg), LPS (200  $\mu$ g/kg) + anti-CD41 Ig, EIII + anti-CD41 Ig, and EIII-SNPs + anti-CD41 Ig]. N = 6. \*\*  $P < 0.01$ , vs. vehicle control groups; ##  $P < 0.01$ , vs. respective LPS + LPS groups; +  $P < 0.05$ , ++  $P < 0.01$ , vs. respective LPS + anti-CD41 Ig groups. Data are presented as mean  $\pm$  SD.

**Figure S3**



**Figure S3. Images of hemorrhage lesions of mice after different two-hit treatments.** Example images of hemorrhage lesions that were used for calculating the hemorrhage score (Figure S2) in mice. (1) vehicle, (2) LPS + LPS, (3) LPS + anti-CD41 Ig, (4) EIII + anti-CD41 Ig, (5) EIII-SNPs + anti-CD41 Ig. Scale bar: 0.5 cm.

Figure S4



**Figure S4. Comparisons of circulating cytokine IL-1 $\beta$  and TNF- $\alpha$  levels after different two-hit treatments in mice.** Enzyme-linked immunosorbent assay was performed to examine the expression of proinflammatory cytokines IL-1 $\beta$ , and TNF- $\alpha$  in mice groups without (vehicle), and with different 2-hit treatments [LPS (200  $\mu$ g/kg) + LPS (1.2 mg/kg), LPS (200  $\mu$ g/kg) + anti-CD41 Ig, EIII + anti-CD41 Ig, and EIII-SNPs + anti-CD41 Ig]. N = 6. \*\*  $P < 0.01$ , vs. vehicle control groups; ##  $P < 0.01$ , vs. respective LPS + LPS groups; +  $P < 0.05$ , ++  $P < 0.01$ , vs. respective LPS + anti-CD41 Ig groups. TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; IL-1 $\beta$ , interleukin-1 $\beta$ . Data are presented as mean  $\pm$  SD.