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Supplementary Materials for

Synthetic high-density lipoproteins loaded with an antiplatelet drug for efficient inhibition of thrombosis in mice

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The PDF file includes:

Figs. S1 to S5

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/6/49/eabd0130/DC1)

Movie S1

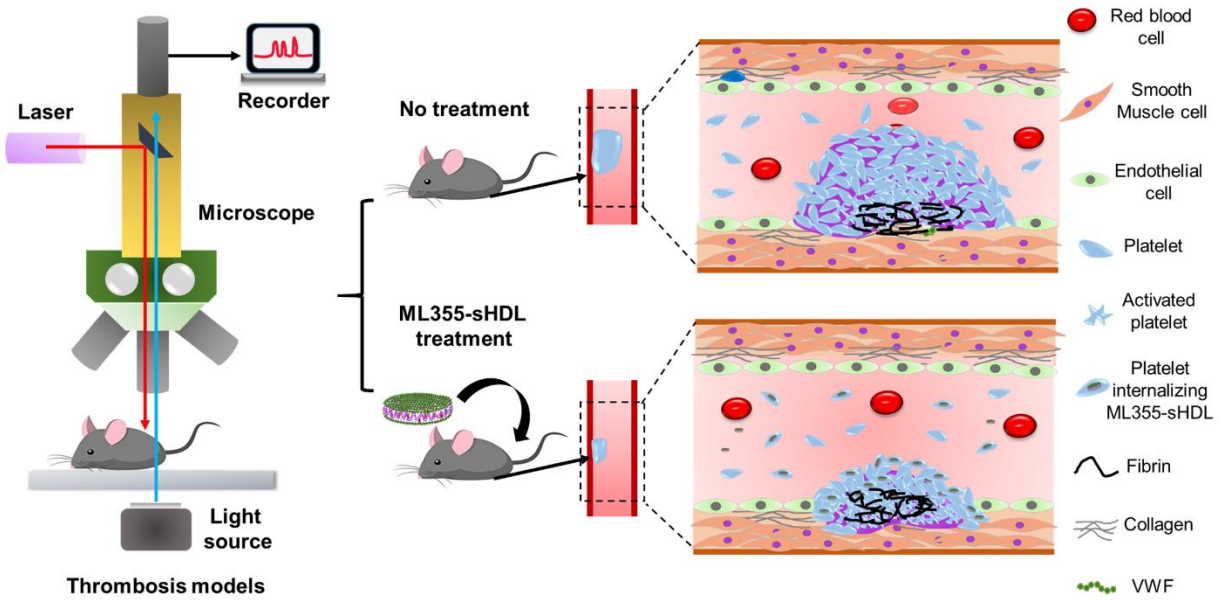

fig S1. Encapsulation of antiplatelet agent ML355 into sHDL (ML355-sHDL) exerts synergistic antithrombotic effects of both ML355 and sHDL, thus efficiently inhibiting thrombus formation.

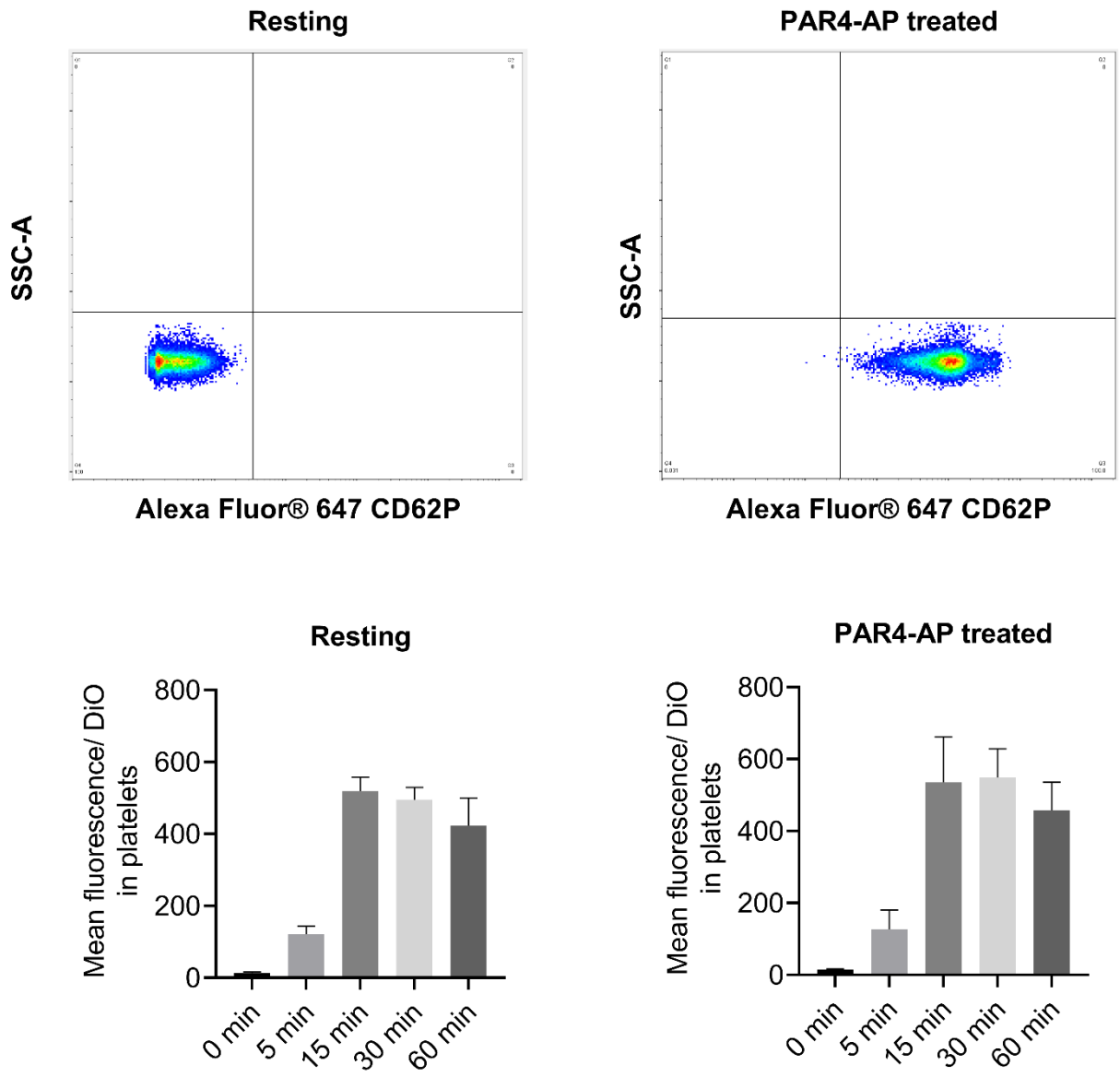

fig S2. The comparison of sHDL uptake by washed mouse platelets (resting) and activated mouse platelets treated with PAR4-AP. Both unstimulated washed mouse platelets and activated mouse platelets pre-treated with PAR4-AP (50 μM) were incubated with DiO-sHDL (sHDL at 50 μg/mL and DiO at 2.5 μg/mL) for indicated lengths of time (5, 15, 30 and 60 minutes) at 37 °C. Platelets were then washed with Tyrode's buffer twice to remove free DiO-sHDL and then fixed with 4% paraformaldehyde, followed by staining with Alexa Fluor 647 conjugated anti-mouse CD41 antibody. Platelet membrane is shown in red and sHDL particle accumulation in platelets is shown in green. Representative flow cytometry scatterplots for Alexa Fluor® 647 CD62P fluorescent intensity in platelets and data analysis of mean DiO fluorescent intensity in platelets by flow cytometry.

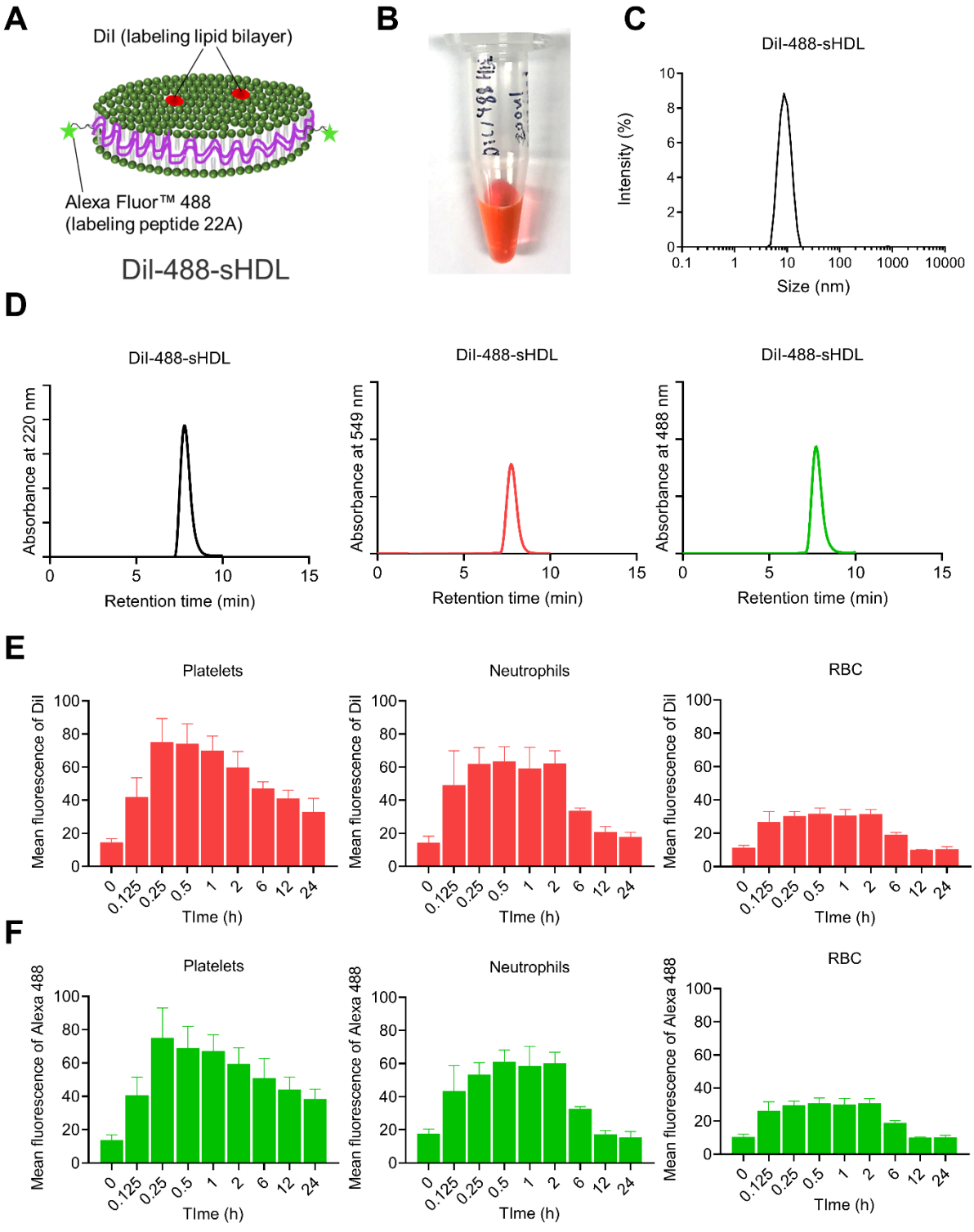

fig S3. The distribution of DiI-488-sHDL in major blood cells. A. Illustration of dual labeled DiI-488-sHDL was prepared by labeling 22A peptide in sHDL with AlexaFluor 488 dye using

Invitrogen protein labeling kit and labeling lipid bilayer in sHDL with cell-labeling fluorophore DiI. (**B**) Photo, (**C**) dynamic light scattering and (**D**) gel permeation chromatography of DiI-488-sHDL at multiple absorbance wavelength 220, 488 and 561 nm, respectively. Photo Credit of DiI-488-sHDL: Hongliang He, The University of Michigan. For investigation of biodistribution of DiI-488-sHDL over time course among major blood cell types, including platelets, neutrophils and red blood cells in mouse, both male and female C57BL/6J mice were intravenously dosed with DiI-488-sHDL (DiI at 0.5 mg/kg and Alexa Fluor 488 at 0.5 mg/kg). At different time points (from 5 minutes up to 24 hours), whole blood were collected and mean fluorescent intensity of both lipid tracer DiI (**E**) and peptide tracer Alexa Fluor 488 (**F**) in each cell type were analyzed by flow cytometry. Data shown as mean ± SD (n = 4).

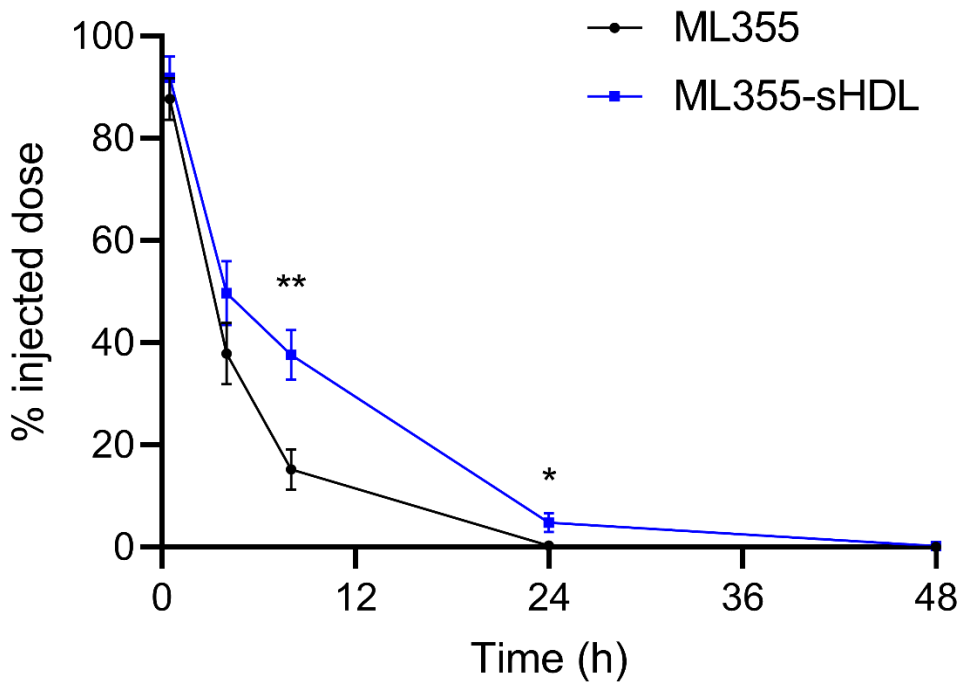

fig S4. **ML355-sHDL increased the blood circulation of ML355 in mice.** A single intravenous injection of ML355 (3 mg/kg) or ML355-sHDL (sHDL at 100 mg/kg and ML355 at 3 mg/kg) in mice. Data shown as mean ± SD (n = 4). *$P < 0.05$ and **$P < 0.01$.

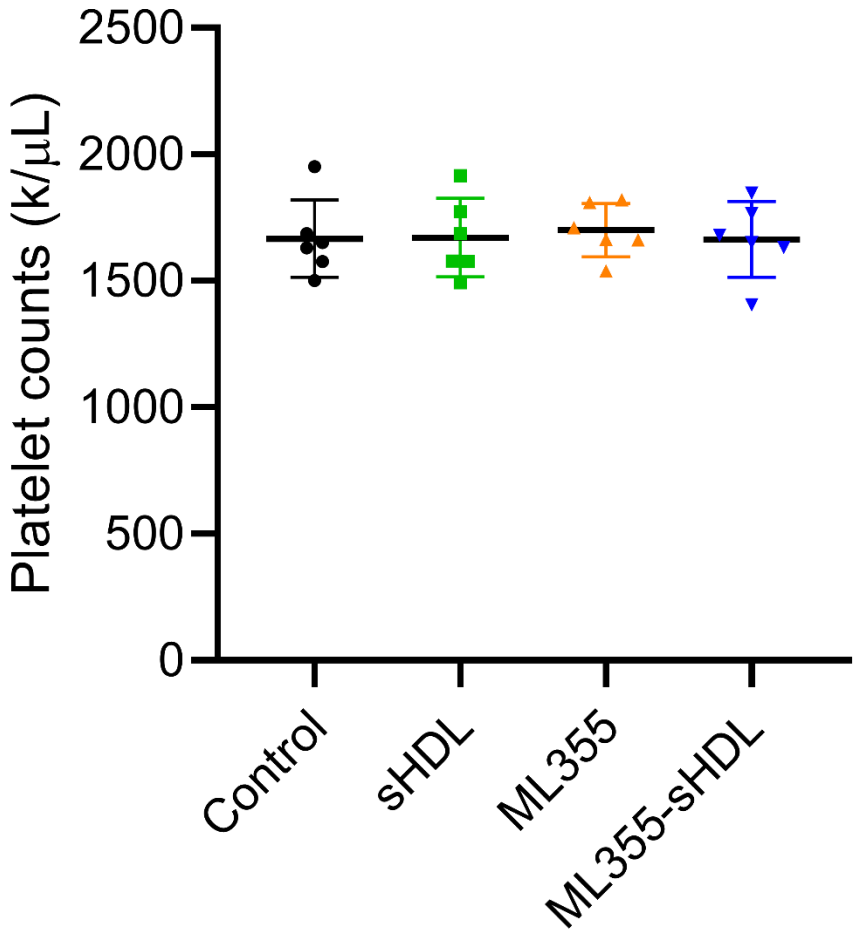

fig S5. **ML355-sHDL treatment do not impact the platelet counts in mice.** Both female and male mice (n = 6) were pretreated IV with saline control, sHDL (50 mg/kg), ML355 (1.5 mg/kg), or ML355-sHDL (sHDL at 50 mg/kg and ML355 at 1.5 mg/kg). After 24 hours, mice were anesthetized by intraperitoneal injection of ketamine/xylazine as described in the methods section. Blood were collected from mice using the lateral saphenous vein. Complete blood counts were performed using a Hemavet 950 analyzer (Drew Scientific Inc., Oxford, CT, USA).