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Supplemental Information

Enhanced Delivery and Effects of Acid Sphingomyelinase by ICAM-1-Targeted Nanocarriers in Type B Niemann-Pick Disease Mice

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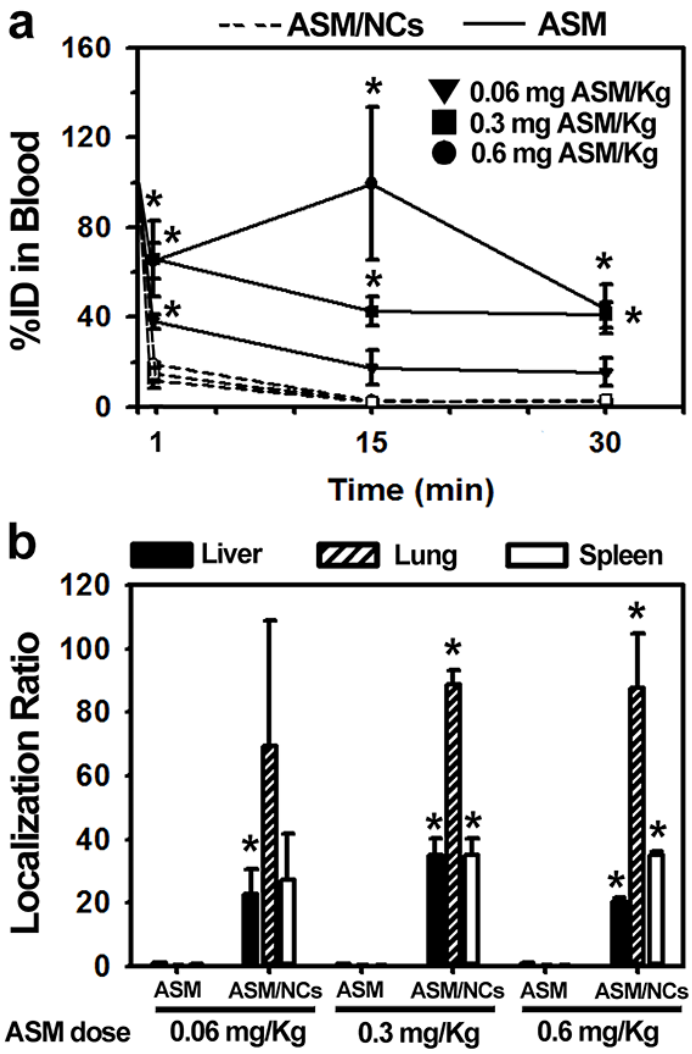
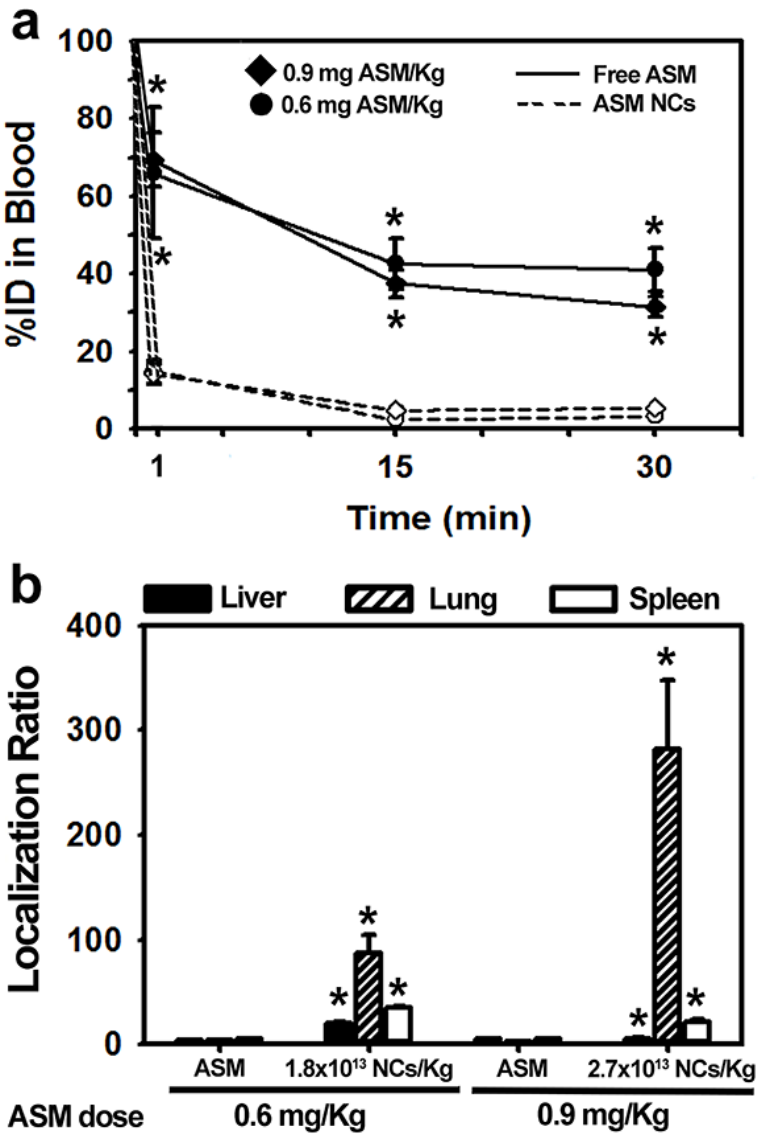
SUPPLEMENTARY FIGURES

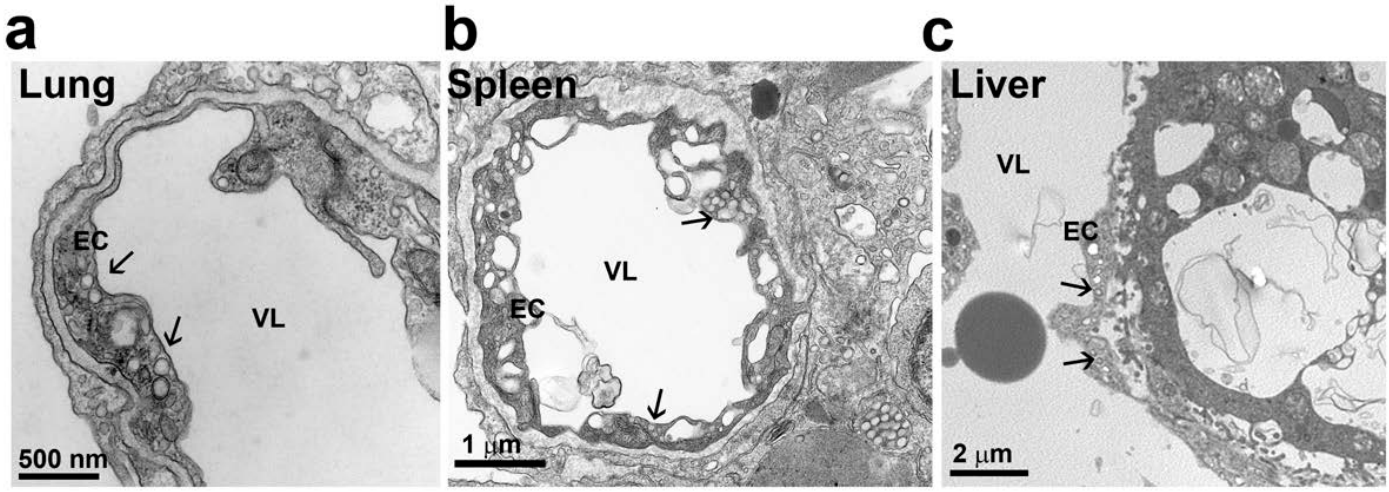


Supplementary Figure S1. Comparative biodistribution of anti-ICAM/ASM NCs containing different ASM loads versus naked ASM. Wild-type (C57BL/6) mice were i.v. injected with anti-ICAM/^{125}I-ASM polystyrene NCs containing 0.06, 0.3, or 0.6 mg ASM/Kg body weight (all at 1.8 x 10^{13} NCs/Kg body weight) or matching doses of naked ASM. (**a**) The ^{125}I content of blood samples taken 1, 15, and 30 min after injection was used to calculate the percent of the injected dose (%ID) in the circulation. (**b**) Similarly, the ^{125}I content in the lungs, liver and spleen isolated at sacrifice 30 min after injection, was used to determine the localization ratio (tissue-to-blood ratio) in these organs (%ID per gram in an organ : %ID per gram in blood). Data are mean ± standard error of mean (n=3-6 mice). *Compares formulations to the lowest ASM load; #compares highest load formulation to the intermediate ASM load ($p \leq 0.1$ by Student's t-test).

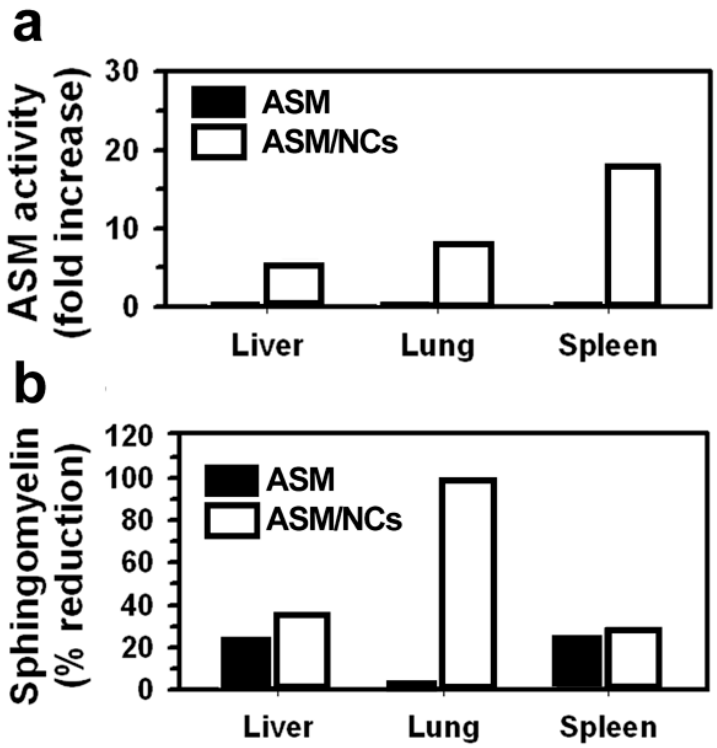


Supplementary Figure S2. Comparative biodistribution of anti-ICAM/ASM NCs at different NC concentrations versus naked ASM. Wild-type (C57BL/6) mice were i.v. injected with anti-ICAM/^{125}I-ASM polystyrene NCs at 1.8 x 10^{13} or 2.7 x 10^{13} NCs/Kg body weight (0.6 or 0.9 mg/ASM/Kg body weight) or matching doses of naked ASM. (**a**) Blood ^{125}I content was used to calculate the percent of the injected dose (%ID) in the circulation. (**b**) Similarly, the ^{125}I content in the lungs, liver and spleen isolated at sacrifice 30 min after injection, was used to determine the localization ratio (tissue-to-blood ratio) in these organs (%ID per gram in an organ : %ID per gram in blood). Data are mean ± standard error of mean (n=3-6 mice). *p≤0.1 by Student's t-test.

Supplementary Figure S3. Movie of anti-ICAM/ASM carriers endocytosis in mice. Time lapse fluorescence microscopy movies of 1 μm, green Fluoresbright®-labeled anti-ICAM/ASM polystyrene carriers (**a**, dark background panels) co-injected i.v. with the fluid-phase marker Texas Red dextran (**b**, light background panels) in wild-type (C57Bl/6) mice. Images from exteriorized mesentery were taken every 30 seconds beginning 15 min after injection. Carriers free flowing in the circulation are not visible. Carriers loosely bound on the endothelial surface are dextran negative and move downstream with time. Carriers that firmly bind on the endothelium after beginning imaging do not move. Presence of punctate dextran co-localizing with carriers indicates carrier endocytosis by the endothelium. Scale bar = 10 μm.



Supplementary Figure S4. Low magnification transmission electron microcopy of mouse organs injected with anti-ICAM/ASM NCs. Transmission electron microscopy of wild-type mouse lungs (**a**), spleen (**b**), and liver (**c**) showing anti-ICAM/ASM polystyrene NCs (arrows) interacting with endothelial cells (ECs), 3 h after i.v. injection. VL = vessel lumen.



Supplementary Figure S5. Theoretically normalized, comparative enzymatic function of ASM delivered in mice as naked enzyme versus anti-ICAM/ASM NCs. (**a**) Fold enhancement in the ASM activity of ASMKO mice injected with recombinant ASM was measured and is shown as described in Fig. 6a for anti-ICAM/ASM NCs, while that of naked ASM has been normalized to the excess enzyme injected as naked versus NC counterparts (correction factor = 3 mg/Kg body weight divided by 0.6 mg/Kg body weight). (**b**) Percent reduction in the sphingomyelin (SM) content in tissue homogenenates from ASMKO mice was measured and is shown as described in Fig. 6b for anti-ICAM/ASM NCs, while that of naked ASM has been normalized to the excess enzyme injected as naked versus NC counterparts (correction factor = 4 injections at 3 mg/Kg body weight (12 mg/Kg) divided by 6 injections at 0.6 mg/Kg body weight (3.6 mg/Kg)).