In vivo siRNA Delivery to Immunosuppressive Liver Macrophages by α-Mannosyl-Functionalized Cationic Nanohydrogel Particles

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Supplementary Information

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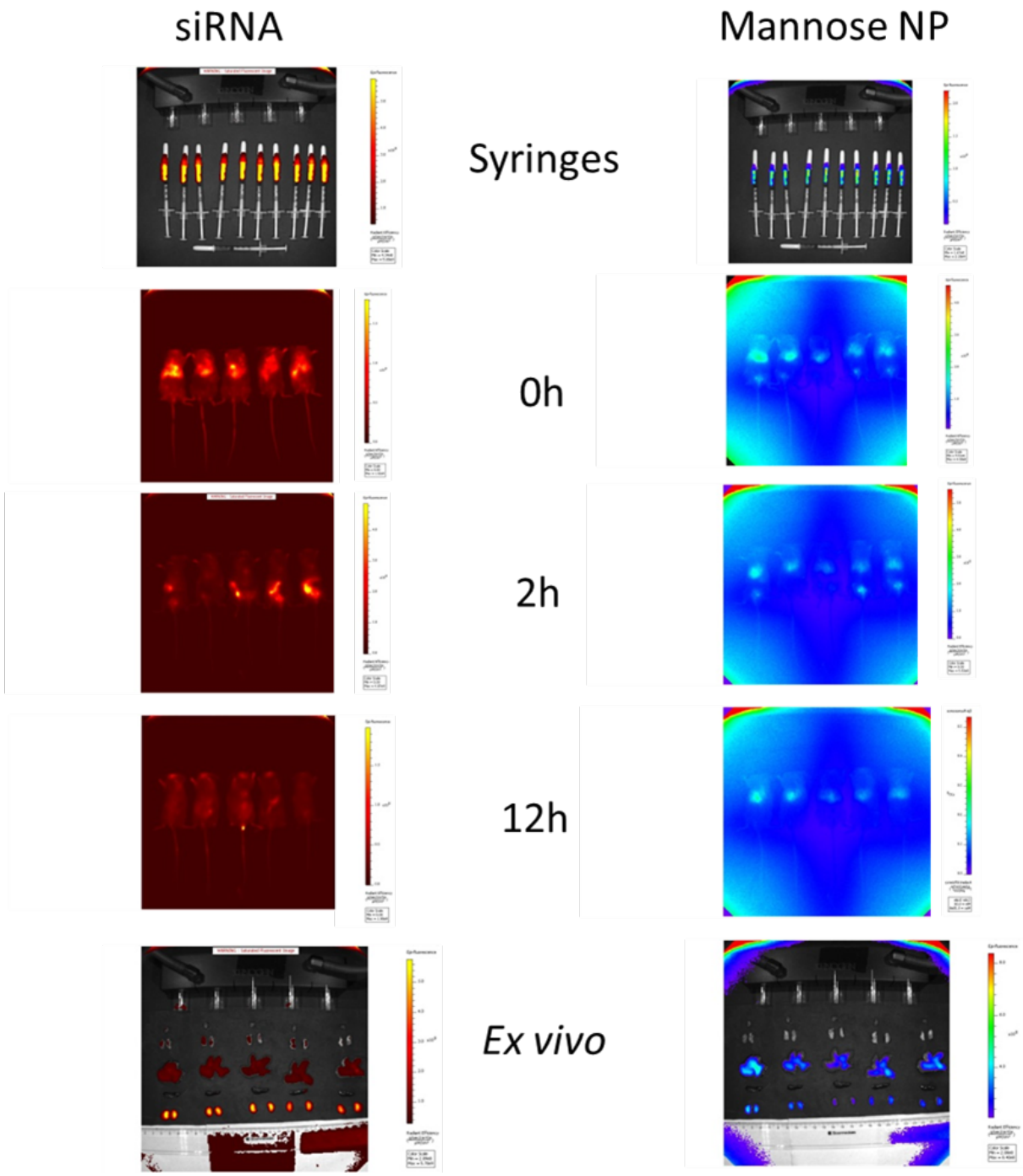
A

SI - Fibrotic mice



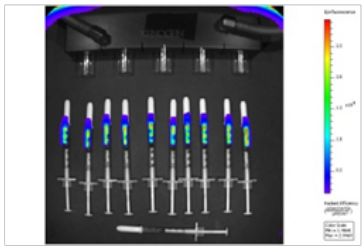
SI - Fibrotic mice

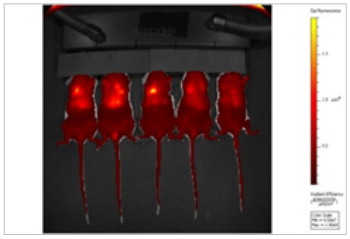
B

siRNA

Non-mannose NP

Syringes





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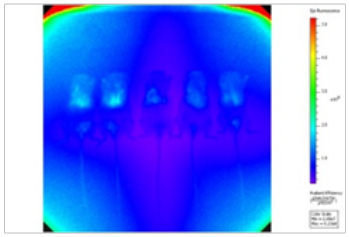

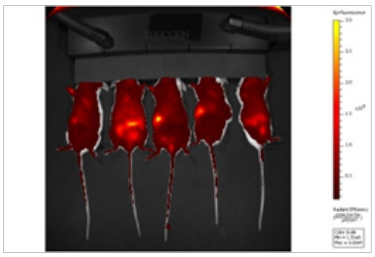

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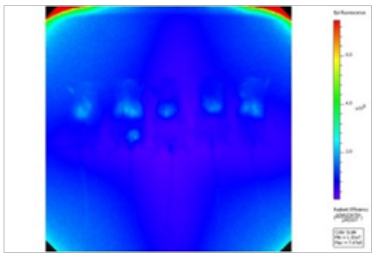
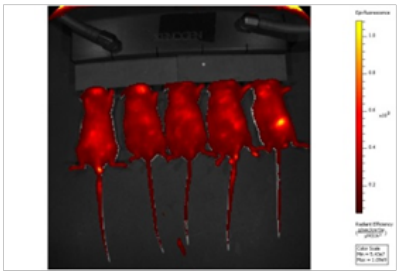
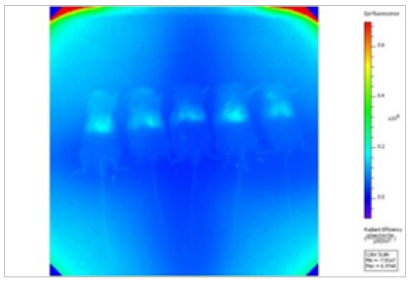
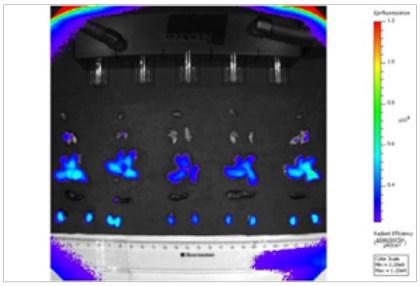




12h



Ex vivo

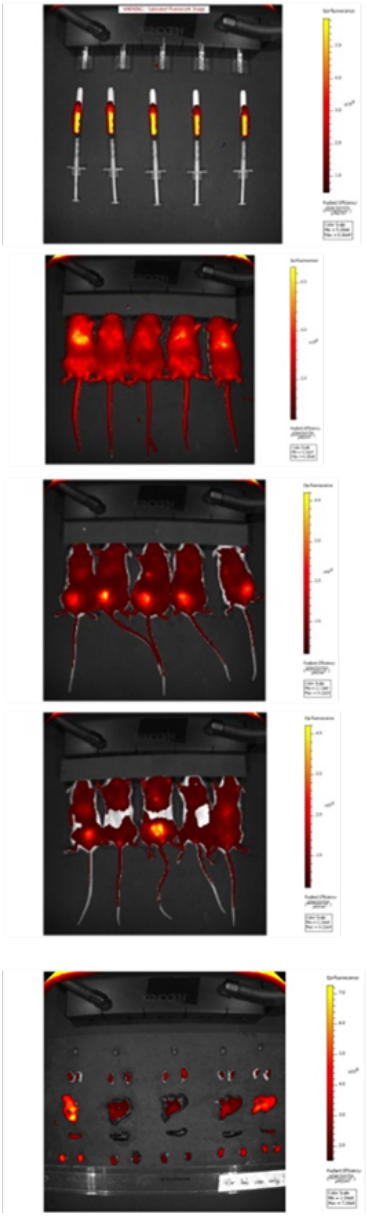


C

SI - Healthy mice

siRNA

Mannose NP



0h

2h

12h

Ex vivo

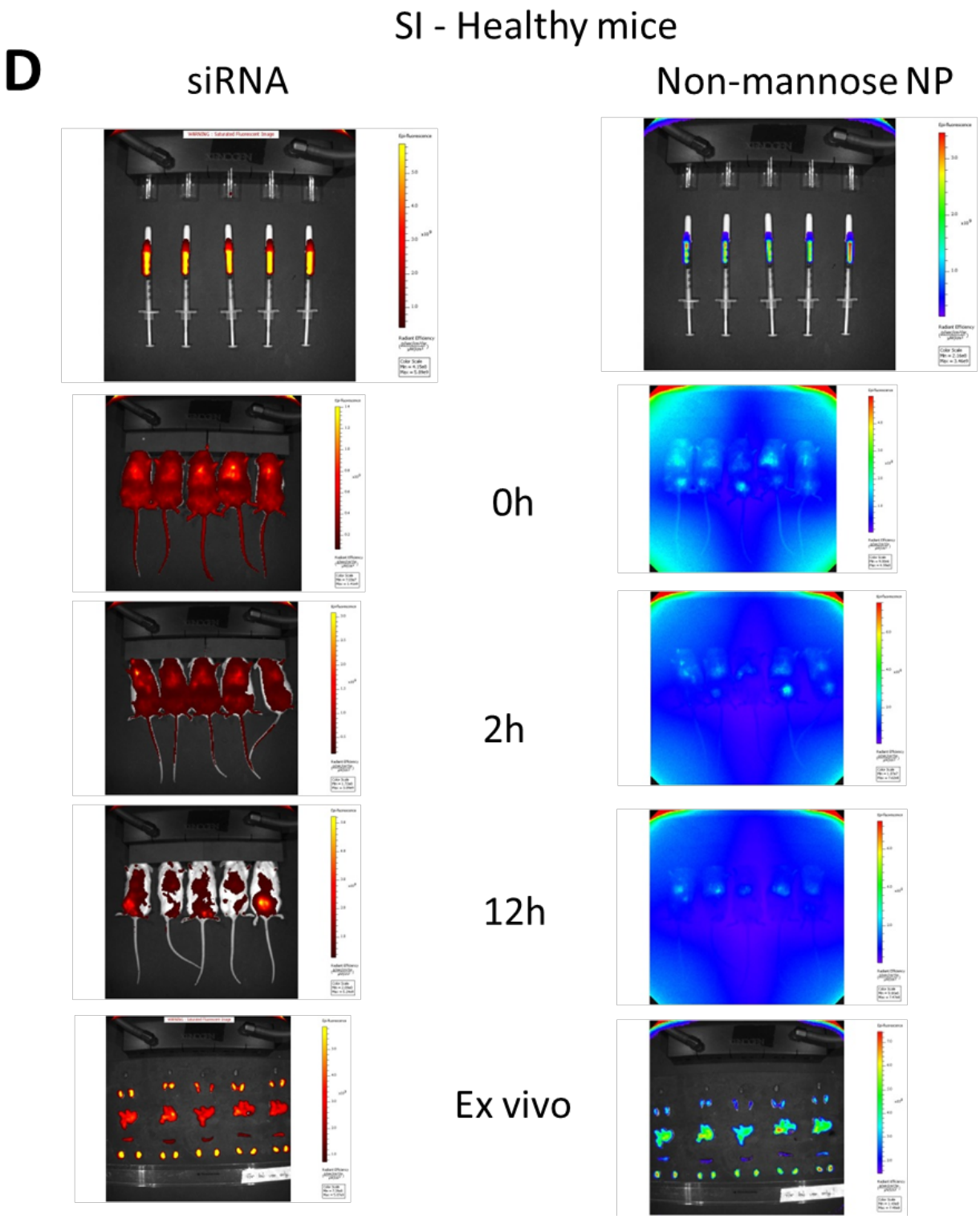


Figure S1. IVIS in vivo imaging of NIR-Cy5-scsiRNA loaded NIR-labeled RS800-(Non-)ManNP at 0, 2 and 12 h after i.v. injection in healthy and liver fibrotic mice (syringes with complexes shown). Ex vivo imaging of the corresponding organs (order: heart, lungs, liver, spleen and kidneys) after 12 h of injection. After i.v. injection, both siRNA cargo and NonNP primarily colocalized in the liver, while breakdown products of carrier and siRNA cargo were mainly excreted via the urinary tract, resulting in a strong fluorescent signal also in the kidneys.

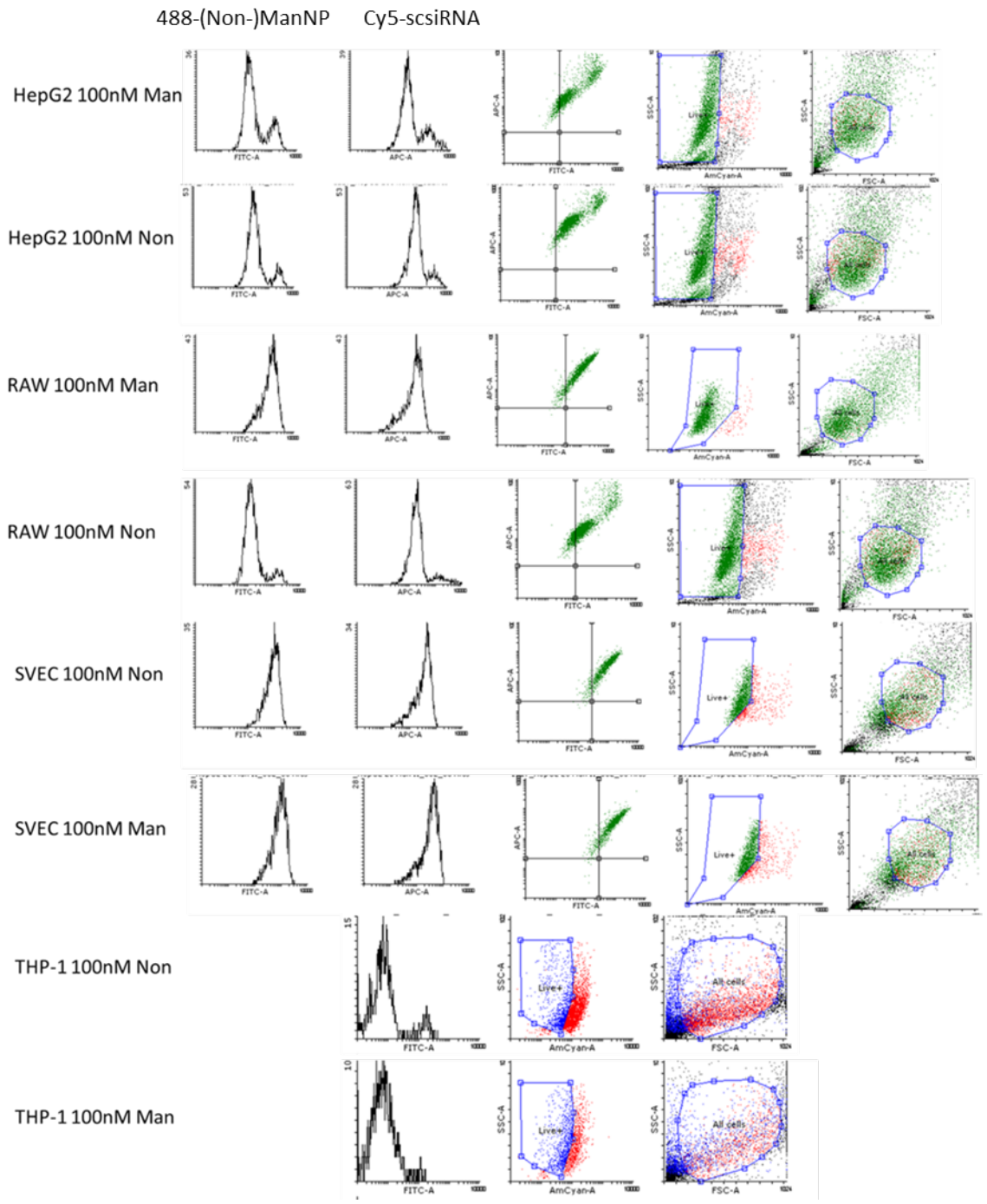


Figure S2. Exemplary dot plots and histograms of the in vitro cellular uptake as determined by FACS analysis: Both Oregon Green labeled (Non-)ManNP (= FITC-A) and Cy5 labelled siRNA (=APC-A) were efficiently taken up in human-hepatocytes (HepG2), -macrophages (THP-1), murine liver endothelial cells (SVEC4-10) and macrophages (RAW).

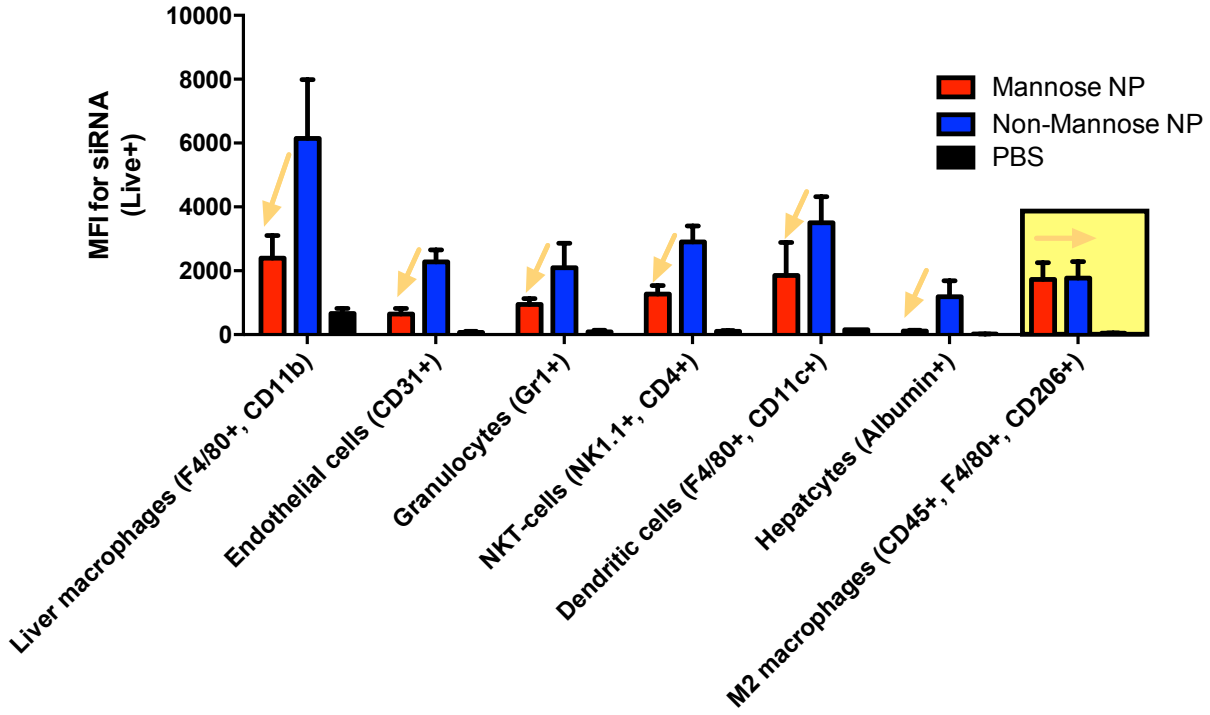


Figure S3. In vivo cellular uptake of RS800-NonNP and RS800-ManNP in (non-)parenchymal liver cells as assessed by FACS analysis of single cell suspension obtained from harvested fibrotic livers. While the non-mannosylated carrier showed a higher overall unspecific uptake in all tested (non-)parenchymal liver cells with no preference for M2 macrophages, uptake of ManNP in CD206+ macrophages remained and increased compared to other cells (indicated by yellow box).

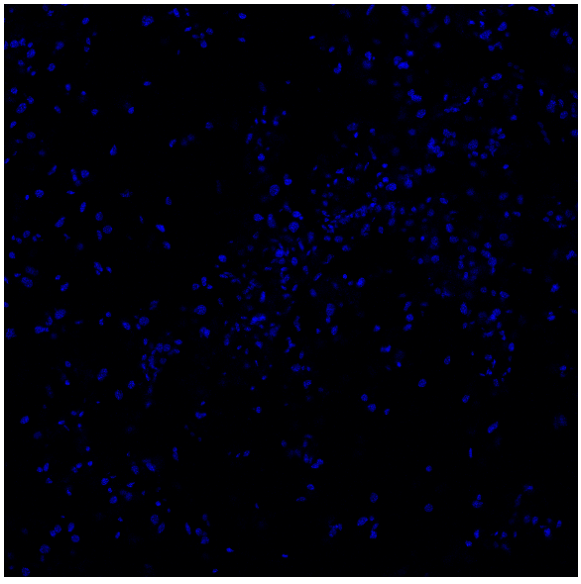


Figure S4. Confocal laser microscopy of a liver cryosection from PBS treated control mice. The control section did not show a fluorescent signal, neither for Cy5-siRNA (red) nor for RS800-(Non-)ManNP.

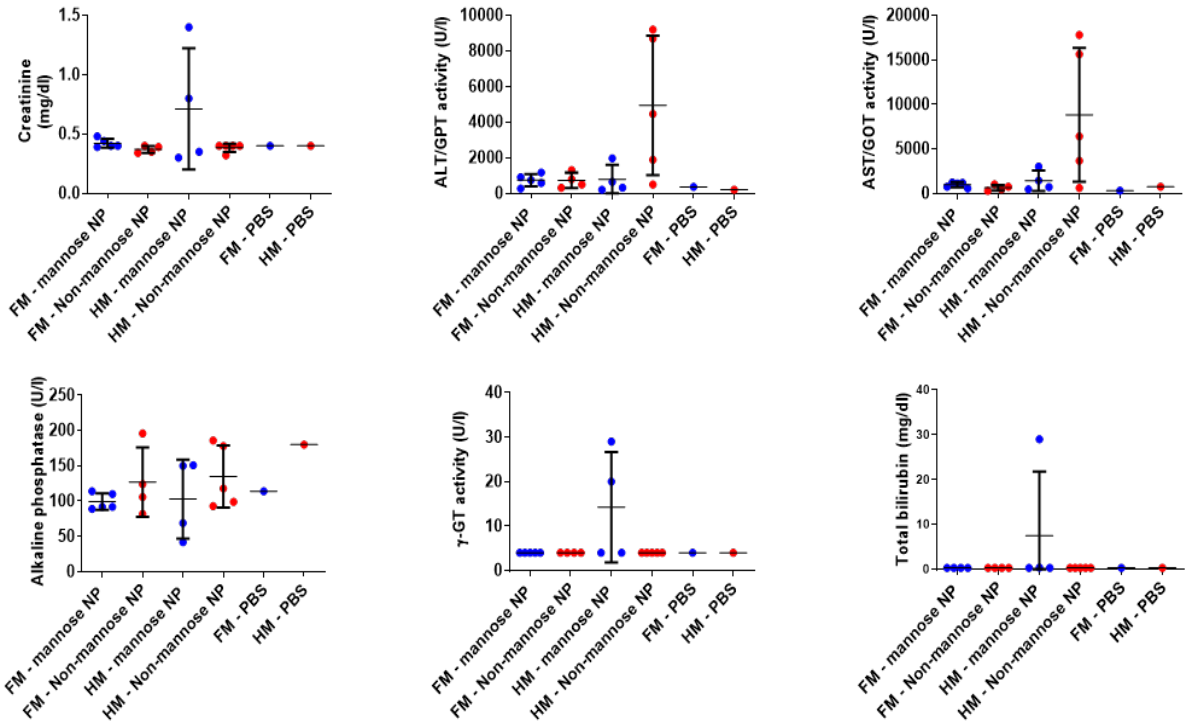


Figure S5. Serum markers of liver and kidney damage from CCl4 liver fibrotic (FM) and healthy (HM) mice treated with Cy5-scsiRNA loaded RS800-(Non-)ManNP (2 mg/kg siRNA) or PBS, respectively. Blood samples were obtained at organ harvest 12 h after i.v. injection of the NP. Normal values and no difference from PBS only injected control mice for liver inflammation (aspartate transaminase, alanine transaminase), cholestasis (alkaline phosphatase, gamma-glutamyltransferase, total bilirubin) and kidney function (creatinine).